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# MEASUREMENT OF SAP FLOW IN CONIFERS BY HEAT TRANSPORT[1]

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An account of the various methods which have been used in an attempt to measure the rate of sap flow in plants has been given by Crafts, Currier and Stocking (2). Methods which require the measurement of the amount of material moving through a known cross-sectional area, or the timing of the movement of some material indicator such as salts, dyes or radioactive material, suffer from the disadvantage that the vessels conveying the sap stream must be severed, either to measure the quantity of sap or to introduce the indicator, causing an unknown disturbance to the initial state. Our experience confirms that on cutting the vessels of a living tree the rate of sap flow always changes greatly in magnitude, often instantaneously.

Heat used as an indicator, however, does not have this disadvantage as it can enter the sap stream by thermal conduction through the walls of the vessels. Measurements using heat have been made by Huber (5) and his school (e.g., Huber and Schmidt (6)), and Dixon (3). Huber's first experiments were made on a tropical liana with such a high rate of sap flow that heat applied for one or two seconds was still recognizable as a pulse at the junctions of a thermocouple 30 cm downstream from the heater. The time till the first appearance of heat at the thermocouple was assumed to be the same as the time taken for the sap to move this distance.

For slower sap speeds it became essential to distinguish between the effect of convection by the moving sap and the transport of heat by thermal conduction. Dixon achieved this by placing the thermojunctions at distances of 1 cm and 2 cm from the heater and considering only cases when the more distant junction at some stage indicated a higher temperature than the nearer one. Huber and Schmidt (6) devised a method for the same purpose in which one junction was 2.0 cm downstream and the other 1.6 cm upstream from the heater. In all these experiments the heater wires and the thermojunctions were laid either on the intact bark or just under the bark on the surface of the sapwood.

There are two fundamental drawbacks to these heat transport experiments. First, it will be shown below that the assumption made in each case that the speed of the sap is identical with that of the heat pulse is not justified. In conifers the sap speed is actually about three times that of the heat pulse. Secondly, comparison between the curves presented for galvanometer deflections versus time and those calculated theoretically shows that, in spite of cotton wool lagging, there was considerable loss of heat; this reduces the usefulness of the results.

A detailed criticism of previous work is given below in the Discussion.

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## THEORETICAL

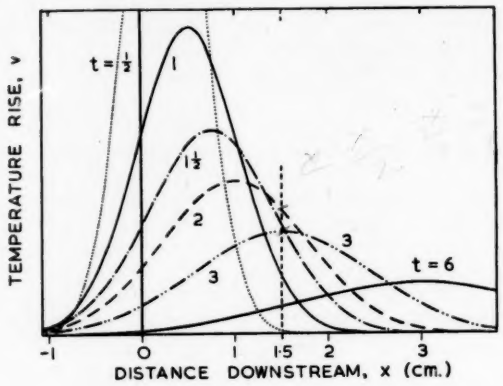
In order to avoid unknown surface losses, the present method uses a heater in the form of a straight line perpendicular to the surface, the temperature being measured at a point far enough below the surface to avoid the effect of these losses. The method of analysis adopted was the usual one of considering an idealized, simplified structure which is amenable to mathematical treatment, and then trying to explain discrepancies between the ideal, theoretical predictions and the experimental results by a reassessment of the simplifying assumptions involved in the mathematics.

For a start ignore the wood substance and consider the xylem to consist of nothing but sap, at rest in the first instance. Assume in these first illustrations that turbulence in the sap can be ignored. Consider a quantity of heat released instantaneously along a straight line in the sap. This heat will gradually diffuse throughout the sap by the process of heat conduction, until eventually all the sap has its temperature raised by the same infinitesimal amount (assuming the xylem to be effectively infinite in extent so that the surface effects can be ignored). The temperature at any point will rise to a maximum and then fall much more slowly to its original value (see fig 2). For a given quantity of heat the temperature at the point at a given time will depend only on the diffusivity of the sap, a quantity which depends on the thermal conductivity K, the density $\rho$, and the specific heat c of the sap. In fact

$$\text{Diffusivity, } k = K/\rho c.$$

Now consider the sap to be moving with uniform, parallel motion perpendicular to the straight line heater. In this case the movement of the pulse of heat will depend not only on conduction but also on convection due to the bodily movement of the sap.

This is illustrated in figure 1, where the abscissa is the direction of motion. (The heater can be visualized as lying along the v-axis. Then, since the temperature distribution is two-dimensional, all points on any line parallel to the heater have the same temperature. Now imagine the heater dimension replaced by the temperature rise ordinates.)



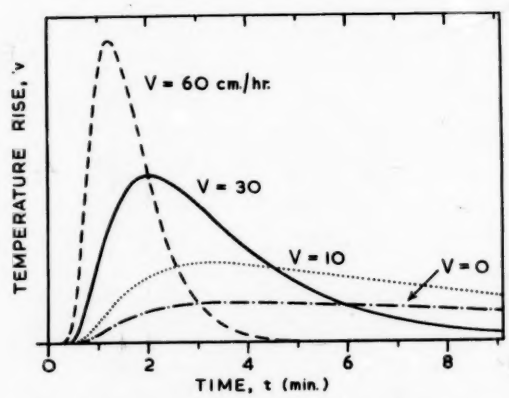




FIG. 1 (*top*). Theoretical temperature distribution at particular times, t (in minutes), after release of heat pulse. Heat-pulse velocity, V = 30 cm/hr. Diffusivity, k = 0.0025 cm²/sec.

FIG. 2 (*bottom*). Theoretical curves of temperature rise against time at the point 1.5 cm downstream from the heater, for heat-pulse velocities, V = 0, 10, 30 and 60 cm/hr. Diffusivity, k = 0.0025 cm²/sec.

As the heat pulse diffuses by conduction it is also moving with the steady velocity of 30 cm/hr. For instance, after three minutes the pulse is cylindrically symmetrical about the ordinate through x = 1.5 cm.

The temperature at a point directly downstream from the heater will rise to a greater maximum than if the sap were stationary, and at an earlier time. It will also return to its original value more quickly. Figure 2, for instance, shows curves of temperature rise against time from the release of the heat pulse, at the point 1.5 cm downstream in a medium with diffusivity, k = 0.0025 cm²/sec for four different velocities, V cm/hr, of the medium. For a given quantity of heat, the temperature at a point at a given time will depend on both the diffusivity of the sap and its velocity. Figure 1 shows that when the effect of conduction is at all comparable with that of convection, the temperature rise at a point will reach its maximum value before the center of the heat pulse reaches the point. This is due to the rapid diffusion of the pulse. Consider, for instance, the situation at x = 1.5 cm; the temperature there is higher at 2 minutes than it is when the center of the pulse arrives at 3 minutes. Consequently the value obtained by dividing the distance to the point by the time to reach the maximum temperature will be an overestimate of the speed of the medium.

So far the xylem has been assumed to consist of nothing but sap. Consider a more realistic model, a material consisting of wood substance perforated by many uniformly spaced, parallel straight tubes through which sap flows at a uniform velocity. The way in which heat flows in this structure will depend on the time taken for any temperature difference between adjacent portions of sap and wood substance to disappear, and this in turn depends on the thickness of the tubes and the separating walls. Fortunately the xylem of a conifer like *Pinus radiata*, on which most of the present work has been performed, is sufficiently fine-structured to allow it to be treated as a homogeneous mixture from the point of view of heat transfer. The wall thickness of the tracheids is about 10 $\mu$ (8) and from the formula, $t = \frac{3l^2}{8k}$ (1, section 36) for the time t seconds required for a slab of thickness $l$ cm and diffusivity k cm²/sec to heat up to the temperature of its surfaces, we have $t \sim 10^{-4}$ seconds, taking the diffusivity of wood substance as 0.004 cm²/sec. Since the time for the temperature to reach a maximum at the measuring point, 1.5 cm from the heater, is always of the order of minutes in practice, this time of 0.1 millisecond is quite negligible.

The case when the non-convecting material is so thick that the time to come to temperature equilibrium with its surroundings is not negligible, is complicated and has not been treated mathematically. This is discussed in the Results in the section on the Absence of Thermal Homogeneity.

The differential equation for conduction of heat in a homogeneous, isotropic solid with diffusivity k is:

$$\frac{\partial v}{\partial t} = k\left(\frac{\partial^2 v}{\partial x^2} + \frac{\partial^2 v}{\partial y^2} + \frac{\partial^2 v}{\partial z^2}\right) \qquad (1)$$

where v is the temperature.

If now a fraction, a, of any plane area perpendicular to the x-axis is occupied by sap streams moving with uniform velocity u parallel to the x-axis, then the layer of material bounded by the planes x and x + $\delta$ x gains heat at a rate per unit area equal to:

$$-au\rho_s c_s \frac{\partial v}{\partial x} \delta x$$

where $\rho_s$ and $c_s$ are the density and specific heat of sap. Inclusion of this term in the derivation of equation 1 yields the differential equation for combined conduction and convection:

$$k\nabla^2 v = \frac{\partial v}{\partial t} + au \frac{\rho_s c_s}{\rho c} \cdot \frac{\partial v}{\partial x} \qquad (2)$$

where k, K, $\rho$ and c refer to the wet wood, i.e., the wood substance together with the stationary and the moving sap. For rising sap the positive direction of x (and u) is vertically upwards.

Dry wood is actually not isotropic, but has a greater conductivity along the grain ($K_x$) than across the grain ($K_y$ and $K_z$). Wet wood may also be anisotropic although probably to a smaller extent. This can be allowed for (1, Section 13) by putting $y_1 = y\sqrt{K_x/K_y}$ and $z_1 = z\sqrt{K_x/K_z}$, which leaves equation 2 unaltered in form.

Equation 2 is of the same form as the equation for pure conduction in a medium moving in the x-direction with velocity:

$$V = au \frac{\rho_s c_s}{\rho c} \qquad (3)$$

(7); that is, the stationary wood and the moving sap together act like a single medium moving at a speed defined by V. This speed is less than that of the sap itself; it is in fact a weighted average of the velocities of the sap and the stationary wood substance. This is not altogether surprising when we consider that in the wood alone the heat pulse would remain stationary as it diffused, while in the sap alone the pulse would move at the same speed as the sap.

The solution for an instantaneous line source of heat along the z-axis, which is relevant to the present method, has been given for the case of a non-moving medium (1, Section 103):

$$v = \frac{Q}{4\pi kt} \exp\left[-\frac{x^2 + y^2}{4kt}\right]$$

(Q is defined to be the temperature to which the amount of heat liberated per unit length of the line would raise unit volume of the substance. This means, when c.g.s. units are used, that $Q\rho c$ cals/cm are released along the line.)

From this we can write the corresponding solution of equation 2:

$$v = \frac{Q}{4\pi kt} \exp\left[-\frac{(x - Vt)^2 + y^2}{4kt}\right] \qquad (4)$$

It will now be seen that figures 1 and 2 also apply to this model for green wood as well as to the case of xylem consisting of sap alone, and these figures were in fact calculated from equation 4. The diffusivity is now that of green wood, and may be expected to lie somewhere between the values 0.0014 and 0.004 cm²/sec, the approximate values for sap (or water) and dry wood substance respectively. The diffusivity will not be known in any particular case since it depends on the moisture content of the sapwood and possibly on other factors.

Thus in general there are two unknown quantities which specify the nature of the heat flow, the diffusivity which is a measure of the heat conduction, and the heat-pulse velocity which measures the heat convection. The latter in turn must be related to the actual speed of the sap flow.

Even with considerable experience it is very difficult to make more than a rough guess at the diffusivity and the velocity associated with a temperature-rise versus time curve simply by visual inspection. Figure 4 gives some idea of the range of pairs of values for velocity and diffusivity which all give curves with the same time for maximum temperature rise at a given point, the sharper curves being associated with faster convection and lower diffusivity. The actual magnitude of the maximum temperature rise is different for each of these curves, but the temperature scale has been arbitrarily adjusted in each case to make all the curves pass through the same maximum point.

It is impractical to use the numerical value of the temperature rise for measuring purposes, owing to the difficulty of releasing an exactly known quantity of heat in the pulse, and of accurately calibrating the temperature measuring device. Instead formulae are used which enable the heat-pulse velocity and the diffusivity to be determined in terms of the relative temperature rises at known times. The most useful formula, which uses any three times such that the middle one is the average of the other two, will now be derived. Other formulae are derived in the appendix.

First eliminate Q, by substituting two points on the curve, ($v_1$, $t_1$) and ($v_2$, $t_2$), in equation 4 and dividing:

$$\ln\left(\frac{v_1 t_1}{v_2 t_2}\right) = -\frac{(x - Vt_1)^2 + y^2}{4kt_1} + \frac{(x - Vt_2)^2 + y^2}{4kt_2}$$

This may be simplified to give:

$$\ln\left(\frac{v_1 t_1}{v_2 t_2}\right) = \frac{(t_1 - t_2)}{4kt_1 t_2}(x^2 + y^2 - V^2 t_1 t_2) \qquad (5)$$

A similar equation may be written for points ($v_3$, $t_3$) and ($v_4$, $t_4$) giving a pair of simultaneous equations in k and $V^2$.

If the times $t_1$ to $t_4$ are chosen so that

$$t_2 - t_1 = t_4 - t_3 \text{ and } t_2 = t_3,$$

i.e., if

$$t_1 + t_4 = t_2 + t_3 = 2t_2,$$

the following solutions are obtained:

$$V = 60r\sqrt{\frac{t_1 \log_{10}\left(\frac{v_1 t_1}{v_2 t_2}\right) - t_4 \log_{10}\left(\frac{v_2 t_2}{v_4 t_4}\right)}{t_1 t_2 t_4 \log_{10}\left(\frac{v_1 t_1 v_4 t_4}{v_2^2 t_2^2}\right)}} \text{ cm/hr}$$

$$k = \frac{3.619r^2 (t_2 - t_1)^2 \times 10^{-3}}{-t_1 t_2 t_4 \log_{10}\left(\frac{v_1 t_1 v_4 t_4}{v_2^2 t_2^2}\right)} \text{ cm}^2/\text{sec} \qquad (6)$$

where $r^2 = x^2 + y^2$ and t is in minutes.

More recently charts have been drawn which enable V and k to be read off directly from the ratios $v_2/v_1$ and $v_4/v_1$.

On carrying out experiments on actual trees a number of departures can be expected from the simplified model discussed here. In particular, it is known that as the sap flows through the xylem it follows devious paths from one tracheid to another, and so the assumption of parallel, uniform flow does not hold except as a first approximation. Again, the heater and temperature measuring probe are of finite dimensions and may cause distortions in the flow of the heat and of the sap. Then also, once the heat-pulse velocity has been found there remains the problem of obtaining a numerical value for the fraction a needed to relate this velocity to the actual speed of the sap flow.

## Experimental Equipment

When making measurements on branches or roots under about 2 inches in diameter the simplest and best form of line heater is a length of 26 swg nichrome resistance wire threaded through a fine hole, usually along a diameter. A current of up to 40 amps is passed through this heater for 1 or 2 seconds by means of a 240/24V step-down transformer; the smaller the sap flow, the greater the current required for the same maximum temperature rise at a given point downstream.

For measurements in the sapwood of the trunk, both terminals of the heater must be at one end. The heater in this case consists of a piece of steel wire, which provides strength and a low electrical resistance, inside a tube woven of fiber glass. A piece of nichrome wire welded to the end of the steel returns outside the fiber glass and the whole assembly is bonded with a silicone varnish, the diameter being 2.5 mm. The whole length of the resistance wire must be well exposed on the surface, otherwise the heat-insulating effect of the electrical insulation causes a "tail" on the heat pulse which alters the shape of the temperature time curve to an unknown extent.

The temperature is measured by means of a Philips 83900 3K5 thermistor set in the tip of a 21 swg hypodermic needle, the needle itself forming one lead and the other passing up the hollow core. The thermistor forms one arm of a Wheatstone bridge which is supplied from an oscillator with 1 kc/s A.C. at about 1 volt. The out-of-balance signal is amplified, rectified and recorded on an Esterline-Angus recording milliammeter. The most sensitive range setting normally used gives a full scale deflection for about 0.5° F temperature rise and the heat input is usually chosen to give a maximum temperature rise of this order.

The thermistor probe and the heater are inserted in parallel holes drilled with the aid of a guiding block, the finer drills being simply chisel-ended pieces of blue steel wire. The spacing of 1.5 cm used most of the time is a compromise between the greater signal obtained with a smaller spacing and the associated increase in the percentage of error.

In field equipment under construction, the heating current will be supplied from an accumulator, and battery-powered electronic devices will operate indicating meters instead of a recorder.

Since iron is a much better conductor of heat than wood it might be expected that the probe would cause a local distortion in the temperature distribution in the wood by heat loss to the outside air. This possibility was examined by calibrating the thermistor at room temperature over a range of a few degrees, under three conditions; with the needle completely immersed in water, with 2.2 cm of the needle protruding into the water from the cork which is normally used to cover the exposed part of the needle, and with 1.0 cm of the needle protruding. Compared with the first case there was found to be a reduction in sensitivity of about 1.5 % in the second case, and 2.5 % in the third. Theoretical considerations indicate that a steady rate of heat loss through the needle, proportional to the temperature rise, is established within a few seconds, resulting merely in a slight reduction in the temperature rise scale of the curve, without distortion. This conclusion is supported by the fact that curves for zero sap flow, for which the effect of heat loss through the probe would be greatest (since the temperature rise stays at high values for a longer time), give an average value for the heat-pulse velocity of zero, although the effect of a considerable heat loss would be to increase the apparent value of this velocity because of the increased reduction of the temperature rise ordinate with time.

## Experiments and Results

The experiments to test the validity and usefulness of the above theory fell into two stages. First it was necessary to find whether the curves of temperature rise versus time were in fact of the predicted type, after which it was necessary to find a practical way of deducing the rate of sap flow from the heat-pulse velocity.

Verification Experiments: The first experiments were made on pieces of cut-off branch which, having zero sap flow, eliminated one of the two unknown quantities, and thus facilitated comparison with theory. The experimental records obtained (fig 3 a) were analyzed by applying the formulae to numerous sets of three points on the curve. If each set gave zero for the heat-pulse velocity, and the

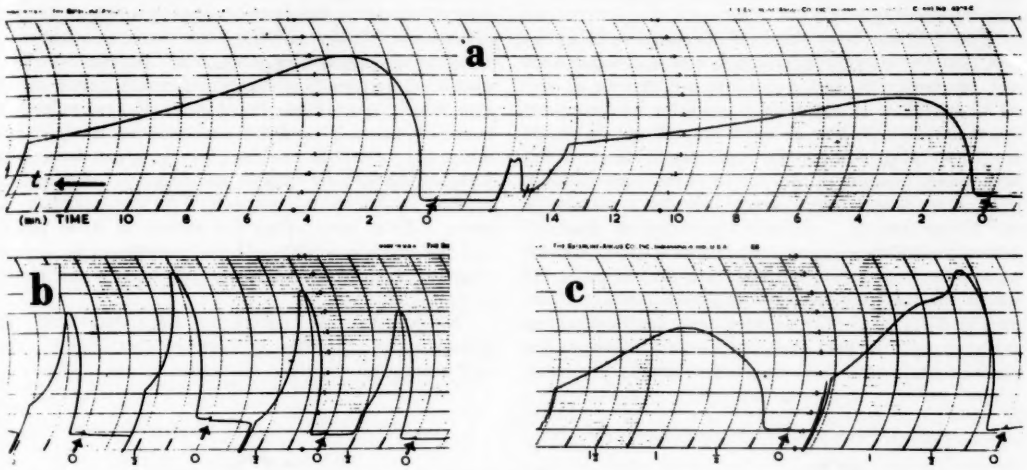



FIG. 3. Experimental records. The vertical arrows show the moment of application of heat. (The heater wire is left permanently in place while a series of measurements is made at one position.) Due to the construction of the recorder the ordinates (temperature) are circular arcs and the time axes (numbered in minutes) are reversed. The break-off at the tail of each curve marks the moment when the chart speed was switched to "slow" to await suitable conditions for another measurement.

(a) Analysis gives: Heat-pulse velocity, $V = 0$; diffusivity, $k = 0.0029$ cm²/sec.

(b) For the right-hand curve analyses range from $V = 314$ cm/hr, $k = 0.00435$ cm²/sec to $V = 283$ cm/hr, $k = 0.0102$ cm²/sec. (For this measuring position when the flow was zero, $k = 0.0020$ cm²/sec.)

(c) Double peaked curves. See text.

same value for the diffusivity this, of course, would indicate exact agreement with theory.

In fact, the values for the square of the heat-pulse velocity in cm/hr lie between $\pm 10$, giving an average value very near the correct value (zero), and indicating a possible error for any one set of points of 3 or 4 cm/hr.

These and later experiments indicate that provided the thermistor is not less than about 0.5 to 1 cm below the surface, the finite dimensions of the piece of wood and the heater and probe do not cause any appreciable deviation from the theoretical case, with its assumption of an infinite medium and infinitesimal experimental apparatus.

A complicating factor which appeared from the start is the variation of the ambient temperature inside the wood. This effect is always present to some extent and is inevitable when first making a measurement in a particular place because drilling the holes upsets the original temperature distribution. When the sap flow is comparatively fast the temperature settles down to a fairly steady value within a few minutes, and fortunately even in cases where the base line temperature continues to fall or rise for a long period it usually does so at a steady rate, which can be extrapolated with considerable accuracy for some minutes beyond the moment of application of heat. Uncertainty in this connection is probably the cause of the spread in the values calculated for the heat-pulse velocity above.

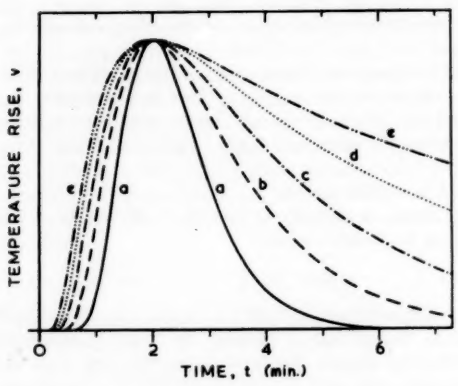
Experiments to test the case of non-zero sap flow were begun on a number of conveniently located conifers, mostly *Pinus radiata*, but the site of the experiments was soon transferred to a plantation of 12-year-old *Pinus radiata* on the sandhills at Woodhill, Auckland, New Zealand. Since it was permissible to mutilate these trees, we carried out dye experiments with acid fuchsin simultaneously with the heat experiments and were thus able to obtain some independent confirmation of the inferences from the heat experiments. The fact that dye experiments cause a major disturbance to the tree and the ultimate destruction of the part measured did not matter of course, in these exploratory experiments.

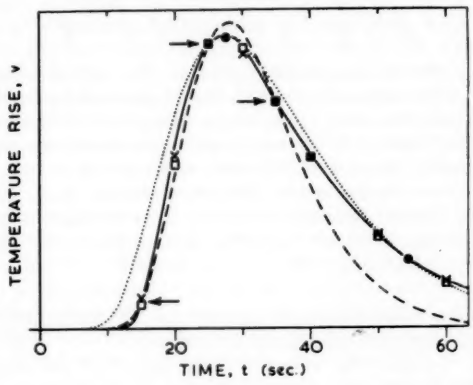
On analyzing the records obtained when the sap is in motion (e.g., fig 3 b) it is usually found that the calculated values for the heat-pulse velocity for particular sets of three points differ by up to 10 % between the greatest and the least, the value decreasing as the point corresponding to the longest time is taken further out on the tail of the temperature-rise versus time curve. (This time is usually not chosen greater than twice the time for the temperature to reach the maximum.) At the same time the calculated values for the diffusivity increase, but even the smallest value is usually considerably greater than the diffusivity measured at the same place when the sap flow is stopped, and this difference is greater the greater the heat-pulse velocity. (It is rather as if, for instance, the rising part of the curve were like that of

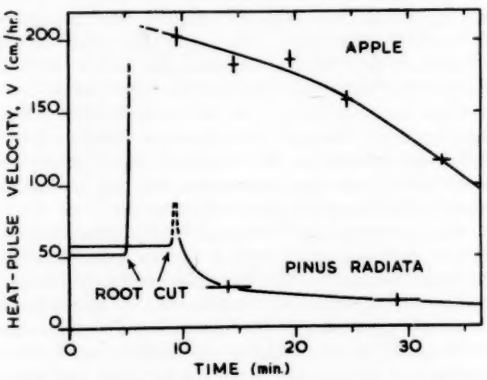
curve b in fig 4 and the falling part like that of curve c in the same figure).

The explanation of these effects seems to be in the fact that the various sap streams whose combined effect produces the recorded curve are flowing at different speeds. If instead of assuming one uniform speed for the sap, it is assumed that all speeds within a certain range are present with equal effect, it has been found possible in the two or three cases tried, to find a theoretical curve which fits the experimental one almost exactly. This is illustrated in figure 5, where the full line is the experimental curve. Analysis of the three points marked with arrows gives 178 cm/hr for the heat-pulse velocity and 0.0031 cm²/sec for the diffusivity. The correct curve for these values is shown by the pecked curve (which intersects the experimental curve at the three analysis points). Similarly analysis of the two points indicated by a solid circle gives heat-pulse velocity 168 cm/hr and diffusivity 0.0059 cm²/sec, the correct curve for these values being shown dotted. Neither of these calculated curves fits the experimental curve at all well, but if a uniform distribution of heat-pulse velocities between 97 and 240 cm/hr is assumed, with a diffusivity of 0.0025 cm²/sec for the green wood, the 8 points marked by crosses are obtained. The points marked by hollow squares, which fit the experimental curve a little better than the crosses at some points and not quite so well at others, correspond to a spectrum between 100 and 238 cm/hr with a diffusivity of 0.0023 cm²/sec.

Notice that the diffusivity associated with the spectrum of velocities is between 0.002 and 0.003 cm²/sec, approximately the same as the values ob-



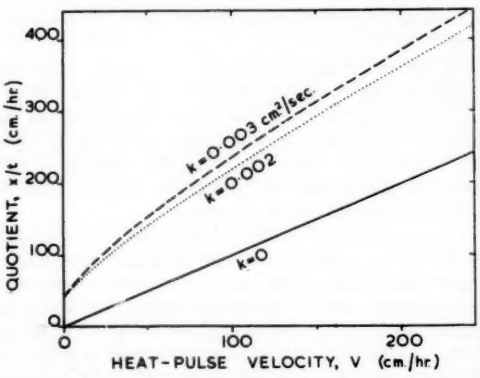




FIG. 4 (*top left*). Theoretical curves for various heat-pulse velocities, V, and diffusivities, k, at 1.5 cm downstream. (a) V = 39.9 cm/hr. k = 0.001 cm²/sec. (b) V = 34.1 cm/hr. k = 0.002 cm²/sec. (c) V = 27.0 cm/hr. k = 0.003 cm²/sec. (d) V = 17.2 cm/hr. k = 0.004 cm²/sec. (e) V = 0. k = 0.00469 cm²/sec.

FIG. 5 (*top right*). For explanation see text.

FIG. 6 (*bottom left*). The change in sap speed on cutting roots.

FIG. 7 (*bottom right*). Theoretical curves for the quotient x/t = distance/time to "first onset" at the point 2 cm downstream against heat-pulse velocity, V, for the cases of diffusivity, k = 0.002 and 0.003 cm²/sec. (Q and v in equation 9 were chosen to make both curves go through x/t = 40 cm/hr at V = 0.) The full line is the curve that would be obtained if there were no conduction, only convection, i.e., if V = x/t.

tained in the absence of flow. The over-simplified analysis assuming a single velocity, attributes the spreading out of the heat pulse to a high diffusivity.

The mathematical expression for the temperature rise in the case where there is a uniform distribution of heat-pulse velocities between $V_1$ and $V_2$ is obtained by integrating equation 4:

$$v = \frac{Q}{4\pi kt} \times \frac{1}{V_2 - V_1} \int_{V_1}^{V_2} \exp\left[-\frac{(x - Vt)^2}{4kt}\right] dV$$

$$= \frac{Q}{4t\sqrt{\pi kt}\,(V_2 - V_1)}\left[\operatorname{erf}\left(\frac{x - V_1 t}{2\sqrt{kt}}\right) - \operatorname{erf}\left(\frac{x - V_2 t}{2\sqrt{kt}}\right)\right]$$

where erf is the error function defined by (1, Section 20):

$$\operatorname{erf} w = \frac{2}{\sqrt{\pi}} \int_0^w e^{-x^2} dx$$

Variations in the rate at which the sap flows through the xylem are to be expected, since the sap as it flows from one tracheid to another may encounter varying degrees of resistance. On a larger scale, dye experiments show that the flow rate may differ greatly between paths separated laterally by a few mm.

Some caution must be exercised, however, in applying this explanation in all cases, for measurements described below in the section on the Absence of Thermal Homogeneity show that a similar apparent spread in speed values can be caused by regions of non-convecting material more than a few mm thick, for which the assumption of thermal homogeneity essential to the mathematical analysis breaks down.

Since the appropriate values for the limits of the spectrum of heat-pulse velocities for a particular recorded curve can only be found by laborious trial and error, the records in practice have been analyzed as if the velocity were single valued. In the cases treated in detail the values obtained were close to the average value for the spectrum (as may be verified in the case of fig 5). These findings on the non-uniformity of the sap flow, however, necessitate a reassessment of whether the "sap speed" is the most useful concept for describing the rate of sap flow. This matter will be discussed below.

Calibration Experiments: The general validity of the theoretical analysis having been established, there remained the problem of relating the heat-pulse velocity to the rate of sap flow. Equation 3 cannot be applied directly because the fraction a is not known; it cannot be deduced from the moisture content of the xylem because only a portion of this moisture consists of moving sap.

During the simultaneous dye and heat experiments discussed above some attempt was made to obtain a direct calibration of the heat transport method against the distance moved by the dye in a known time. Although this procedure would make the present method simply a refinement of the dye method it would still have the great advantage that once a calibration had been obtained for a particular tree, further measurements could be made on the same part of similar trees without disturbing them beyond drilling holes for the heater and probe. There is still the disadvantage, however, that it is not known whether the dye travels as far as the sap before being deposited on the vessel walls, and in any case the dye will only indicate the maximum speed along a given dyed path without giving any indication of the variation in speed in that path.

In actual fact the wide variation over the cross-section in the distance travelled by the dye allowed only a rough estimate of the average sap speed, but it was usually between two and four times the heat-pulse velocity. (This corresponds to a value for the fraction a of the right order, somewhere between 15 and 40 %.) Although this aspect of the experiments was not very useful, some of the results obtained are instructive in showing the kinds of change in the sap flow that may occur when the sap vessels are cut. Two cases are illustrated in figure 6. Both measurements were made on roots, about a foot away from the trunk. A length of split rubber tube was clamped at each end around the root and filled with dyed water, and then the root was cut under the water at a point about one foot below the measuring position.

The case of *Pinus radiata* is typical. After a momentary increase (shown by dye measurements), the flow decreases steadily. The case of apple was the only measurement made on a hardwood. Drought conditions were prevailing and the consequent sap tension, and the removal of the flow resistance at the roots probably explain the fourfold increase in sap speed. It should be noted that this graph shows only relative changes in the sap speed since the ordinate is the heat-pulse velocity.

(The length of the arms of each cross in fig 6 is a rough measure of the uncertainty in the velocity measurements, or the time to take the measurement. The unexpectedly fast flow in the apple root prevented the use of the most suitable recorder chart speed. Note that the faster the flow, the shorter the time for each measurement and the more rapidly they may be repeated.)

Since the experiments at this stage showed, 1st that the sap speed may be far from uniform, and 2nd that some of the analytical precision of the heat transport method of measurement would be lost in attempts at purely empirical calibration, it was questioned whether the sap speed (necessarily an average value) was the best measure of the rate of sap flow. The alternative was to use the volume rate of flow, i.e., the number of cc of sap crossing each sq cm of sapwood perpendicular to the direction of flow in unit time. This concept is well known to physicists as "flux."

It appears that values for sap speeds may be used for two purposes, either to compare rates of sap

flow at different times and different places (in the same tree or different trees), or to relate the sap flow to other numerical quantities such as the rate of evaporation from the leaves, or the moisture intake at the roots. For the first purpose the sap flux is just as useful a measure as the sap speed, and for the second it is more useful because, to relate the sap speed to quantities like the above (evaporation, moisture intake), the speed must first be multiplied by the unknown factor a. For these reasons, and because the relation between the heat-pulse velocity and the sap flux is easily expressed in terms of quantities familiar to the timber technologist, it was decided to use the sap flux as the measure of the sap flow.

The quantity a u in equation 3 is, in fact, the sap flux, and so the equation may be written:

$$\text{Sap flux} = au = \frac{\rho c}{\rho_s c_s} V \qquad (7)$$

The coefficient of V can be expressed in terms of the basic density and the moisture content of the sapwood:

$$\text{Basic density} = \rho_b = \frac{\text{oven dried weight of wood}}{\text{green volume}}$$

$$\text{Moisture content} = m_c = \frac{\text{green weight} - \text{oven dried weight}}{\text{oven dried weight}}$$

It is evident that $\rho$, the density of the green wood, is given by:

$$\rho = \rho_b(1 + m_c)$$

The specific heat is given by:

$$c = \frac{c_w + m_c c_s}{1 + m_c}$$

where

$c_w$ = specific heat of oven-dry wood = 0.33 (4)
$c_s$ = specific heat of sap (water) = 1.00
$\rho_s$ = density of sap = 1.0

On substitution, equation 7 becomes:

$$\text{Sap flux} = \rho_b (m_c + 0.33)V \qquad (8)$$

The moisture content is to be expressed as a decimal fraction (not a percentage) and the density in g/cc. Then if the heat-pulse velocity, V, is in cm/hr the flux is in cc/sq cm/hr.

The verification of equation 8 was carried out in laboratory experiments in which water was sucked through a length of wood by means of a vacuum pump. Roots or slender tree tops were discarded after a brief trial because even with the end surfaces freshly cut the flow decreased too rapidly with time, and varied too much over the cross-section.

With the object of getting a more uniform rate of flow over the cross-section, dowels 2.8 cm in diameter were made from the sapwood of freshly milled pine timber and lacquered on the sides to prevent air leak. Each cross-section contained parts of three annual rings which were about 1 cm wide. These dowels were found to have the added advantage that the flow rate decreased only very slowly with time, allowing greater control over the experiments.

The flux was measured by dividing q, the volume rate of flow of water through the dowel (in cc/hr) by the area of the convecting cross-section (in sq cm). The dye—in these experiments basic fuchsin decolorized with sodium metabisulphite—now served simply to define this convecting area.

Very dilute dye was run continuously while simultaneous measurements of the volume flow rate, q, and the heat-pulse velocity, V, were made with different rates of flow. These two measurements, q, and V, were found to be closely proportional, confirming that the heat-pulse velocity is indeed a measure of the rate of sap flow.

In verifying equation 8 the uniformity of flow over the cross-section was first checked by taking a series of heat-pulse velocity measurements with the tip of the thermistor probe at intervals of about 3 mm along a diameter of the dowel. (These measurements were useful only when the depth was greater than about 5 mm since the effect of heat loss at the surface becomes noticeable at shallower depths.) On the occasion when the uniformity was carefully checked in this way, the cross-section was found to be completely dyed except for two strips 1 or 2 mm wide at the late wood. In this case and in others where the dye pattern was similar, the formula of equation 8 was found to be true within the experimental error (a few percent). It does not seem worthwhile to quote the numerical values here since in these laboratory experiments the rates of flow were about four times greater than the highest measured in a living tree (the latter being about 90 cc/sq cm/hr).

Absence of Thermal Homogeneity: When there are bands of non-convecting material wide enough to cause the condition of thermal homogeneity to break down, the results are not so easily interpreted. This situation has not been treated mathematically but a qualitative picture can be obtained from simple reasoning. Consider a non-convecting region abutting on to the thermally homogeneous region at a plane parallel to the direction of sap flow, and consider a cylindrical pulse of heat perpendicular to the boundary. As this pulse moves forward with the heat-pulse velocity of the convecting region, the effect of the non-convecting region is to retard the pulse near the boundary, while at the same time the pulse extends with further retardation into the non-convecting region. For, as the pulse moves forward due to convection, heat will be transferred by conduction from the forward part into the non-convecting region, while

at the trailing edge of the pulse some of the heat deposited previously will be conducted back into the convecting region.

These considerations are supported by experiments on a dowel which contained both sapwood and heartwood running parallel to the length of the dowel. The zero flow in the heartwood was shown by complete absence of dye. Measurements made with the thermistor at various points along a diameter perpendicular to the boundary of the two regions gave non-zero values for the heat-pulse velocity at depths up to 7 mm into the heartwood, the values decreasing approximately linearly with depth. Analysis based on the assumption of a spectrum of velocities indicated a wide spectrum with values ranging down to zero.

It is possible that the uniform speed analyses of this case may be taken at their face value, on the interpretation that the heat transport method measures the sap flux over an area about 2 cm across centered on the thermistor (when this is spaced 1.5 cm from the heater), and that there is still some flux across this area as long as the thermistor is less than about 1 cm into the heartwood.

In the same dowel there was a non-convecting band about 5 mm wide flanked by dyed regions. Here curves were obtained with two peaks corresponding to heat-pulse velocities of about 225 cm/hr and 65 cm/hr, the relative magnitudes of the peaks changing when the thermistor was moved. It would appear that the water in the neighboring regions was moving with quite different velocities—certainly this is further evidence that a moving pulse extends beyond the actual region of flow (fig 3 c).

## Discussion

It is clear that the findings described in this paper necessitate a reinterpretation of all previous sap flow measurements made by heat transport methods. Previous workers have limited themselves to finding the heat-pulse velocity, assuming this to be the same as the sap speed, and have not always been successful even in obtaining this velocity. Huber appears to be the only one to attempt calibration experiments (6) when he compared the results of his "compensation method" with the rate of flow of water through twigs, measured in drops per second. At best such experiments could show no more than a proportionality between the heat flow measurements and the volume rate of sap flow; there is no question of an absolute comparison with sap speed. Huber simply assigned to a particular flow rate (in drops/sec) the speed indicated by his earlier heat transport method, which itself was based on the assumption that the time to the first appearance of a temperature rise at the nearest thermojunction to the heater was the time for the sap to travel the distance between heater and junction.

Since the methods used by Huber and his school (the originators and principal users of heat transport methods) are based on this concept of the "first onset" of heat, it is instructive to consider the theoretical connection between the heat-pulse velocity and the time of first onset; that is, the time, t secs for the temperature at a point distant x cm directly downstream from the heater to rise by a certain amount, v° C. This is easily obtained from equation 4. Admittedly this equation applies to the case of homogeneous thermal mixing and absence of external heat loss, whereas most of Huber's experiments were performed on hardwoods with comparatively large sap vessels and with measuring devices just under the bark. But it is almost certain that the appropriate equation for all cases of convective heat diffusion will include the exponential term in equation 4, which is the most important term in the present connection. One finds that:

$$V = \frac{x}{t} - \sqrt{\frac{4\alpha k}{t}} \qquad (9)$$

where

$$\alpha = \ln\left(\frac{Q}{4\pi k t v}\right)$$

The expression $\alpha$ includes all the parts of equation 4 not in the exponential term, and being logarithmic does not vary nearly so rapidly as do the quantities t, v, Q and k themselves. It can be seen that the square root term, which allows for the effect of heat conduction, is not simply a constant to be subtracted from $\frac{x}{t}$ in the case of slow sap speeds and ignored in the case of faster flow. In fact this "correction term" increases with increasing flow rate.

Equation 9 can be written in another way:

$$x = Vt + \sqrt{4\alpha k t}$$

which shows the separate effects of convection and conduction more clearly. It gives the distance, x cm which a temperature rise of v° C has reached after t secs. Convection has accounted for a distance Vt as expected, but conduction has added a distance roughly proportional to $\sqrt{t}$. The value of $\alpha$ decreases with the time, t, until it eventually becomes negative at which stage there is no real value for x. This is because the heat pulse has become so diffused that even at its center the temperature rise is less than the threshold temperature rise, v° C.

When $\frac{x}{t}$ is plotted against heat-pulse velocity, V, using equation 9 one obtains curves such as those in figure 7, which are concave towards the V axis. It is interesting that when Huber plotted his "uncorrected speed," $\frac{x}{t}$ (using his "compensation method"), against the flow rate in drops/sec he found in most cases a similar concavity. Huber indicated as a possible explanation, the increase in convecting area with increasing flow rate, but he did not verify quantitatively that this effect was sufficient to account for the curva-

ture. It is not even certain without further investigation that Huber's assumption, that the time of "lower reversal" in his compensation method corresponds to a time of first onset at the downstream junction, is justified. But if this is correct, as seems quite likely, then I consider it almost certain that the main reason for the curvature lies in the variable correction term in equation 9. That Huber himself was unconvinced of the validity of his correction procedure is shown by the concluding sentence of his discussion, namely: "It is clear that theoretically a similar correction (i.e., subtraction of a constant to allow for heat conduction) is needed for all speed values at 4 cm spacing (of heater and nearest junction), but at higher speeds one can generally refrain from making this correction if results of both methods are not to be directly compared with one another."

Analysis of some of Huber's results relating to hardwoods, on the basis of the present theory which was developed for the case of thermally homogeneous softwoods, shows considerable discrepancies as is to be expected. In particular it is found that the magnitude of the heat pulse has decreased much more than the theory predicts in the 4-cm distance. This is probably due to some or all of the following reasons: heat loss to the outside air, the fact that much of the heat remains as a stationary diffusing pulse in the wood and only a portion is carried onward by convection by the sap streams, and thirdly, loss of heat from these sap streams to the unheated walls of the vessels through which they pass. Further research both experimental and theoretical is required before the correct formulae can be found; in the meantime no exact prediction can be made concerning the value of $a$ in the case of hardwoods, and hence of the correction term to be subtracted from $\frac{x}{t}$ to obtain the heat-pulse velocity. One can only be certain that the values given by Huber as "sap speed" are really an overestimate of the heat-pulse velocity.

One aspect of the analysis of Huber's results adds confirmation to the present theory. The time of "lower reversal" in the case of zero flow leads to a value for the diffusivity, and the theory is applicable to all kinds of wood, or indeed to any material. Huber's figures indicate diffusivities from 0.002 to 0.0044 $cm^2$/sec, typical values for green wood.

Regarding Dixon's method of measurement (3) one can say that for softwoods in the absence of heat loss the estimate of heat-pulse velocity would be correct, but in the case of heat loss or in hardwoods the correct interpretation is uncertain.

Over and above all this there remains the fact that the actual sap speed exceeds the heat-pulse velocity, in the case of softwoods by perhaps as much as 400 %, but for hardwoods probably by a much smaller amount. It may be that some of Huber's figures are fairly accurate if his overestimate of the heat-pulse velocity should coincide with the excess of sap speed over heat-pulse velocity, but no reliance can be placed on this. His results, of course, remain very valuable as a measure of relative rates of sap flow.

It might be thought that the simplicity of the "first onset" formula, equation 9, would make it more useful in practice than our analysis formulae. With our analysis charts, however, each curve can be analyzed in a few minutes, and this method has the following advantages. In the first place, as previously mentioned, there is no need to measure the quantity of heat in the pulse or to calibrate the thermistor equipment. Secondly, it is possible to find both the heat-pulse velocity and the diffusivity from a single measurement—there is no need to make a measurement with zero flow in order to find the diffusivity. Our present methods allowed an accurate check on the validity of the theory and revealed the non-uniformity of the sap flow discussed earlier in the section on Verification Experiments, which would otherwise have gone unnoticed. There may, however, be occasions when the first onset formula would be useful, such as when a large number of successive measurements are to be made at one measuring point where the variation in apparent diffusivity with heat-pulse velocity has already been found.

## Summary

Apparatus incorporating a thermistor to measure temperature rises of less than 0.5° F is used to measure the volume rate of sap flow through an area of a few sq cm in the xylem of a conifer.

The method of measuring sap flow by heat transport is put on a sound theoretical basis, confirmed by experiment. The theory shows that the assumption made by previous workers, that the velocity of a heat pulse through the xylem is identical with that of the sap, is not correct. This finding applies to all trees but the exact mathematical relationship derived here applies only to softwoods, which have a comparatively homogeneous xylem.

The present experimental method gives a measure of both the heat conduction and the convection (heat-pulse velocity) from the temperature-time curve of a single heat pulse. If only relative sap flow rates are required, the heat-pulse velocity is an adequate measure, and the only injury to the tree consists in drilling two fine holes. If, however, the actual sap flux in cc/sq cm/hr is desired, a sample of the sapwood must be removed to measure its basic density and moisture content. With the thermistor spaced 1.5 cm from the heater this sap flux is an average over an area about 1.5 cm to 2 cm across, centered on the thermistor.

The apparatus promises to be a useful tool in sap flow research generally. The present experiments, for instance, which were designed simply to verify the theory, have shown the degree of dispersion in the rate of sap flow and the extent of the change in flow on severing the sap streams.

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## Appendix

I. Other Analysis Formulae: If on p. 387 the times $t_1$ to $t_4$ are chosen so that:

$$\frac{t_2 - t_1}{t_1 t_2} = \frac{t_4 - t_3}{t_3 t_4} \text{ and } t_2 = t_3$$

i.e., if

$$\frac{1}{t_1} + \frac{1}{t_4} = \frac{1}{t_2} + \frac{1}{t_3} = \frac{2}{t_2},$$

the following solutions are obtained:

$$V = 60r \sqrt{\frac{\log_{10}\left(\frac{v_1 t_1 v_4 t_4}{v_2^2 t_2^2}\right)}{t_2\left\{t_4 \log_{10}\left(\frac{v_1 t_1}{v_2 t_2}\right) - t_1 \log_{10}\left(\frac{v_2 t_2}{v_4 t_4}\right)\right\}}} \text{ cm/hr} \quad (10)$$

$$k = \frac{1.8095 r^2 (t_2 - t_1)(t_4 - t_1) \times 10^{-3}}{t_1 t_2\left\{t_1 \log_{10}\left(\frac{v_2 t_2}{v_4 t_4}\right) - t_4 \log_{10}\left(\frac{v_1 t_1}{v_2 t_2}\right)\right\}} \text{ cm}^2/\text{sec}$$

where again t is in minutes.

V and k can thus be obtained in terms of ratios of the temperature rises at three times such that the middle time is either the arithmetic mean or the harmonic mean of the other two.

If the point of maximum temperature rise ($v_m$, $t_m$) is used, only one other point of the curve is required. For, by differentiating equation 4 with respect to t and equating the result to zero, we get:

$$4kt_m = x^2 + y^2 - V^2 t_m^2 \quad (11)$$

Substituting $t_1 = t_m$ and $t_2 = pt_m$, where p is any number, in equation 5:

$$\ln\left(\frac{v_m}{pv_2}\right) = \frac{1 - p}{p} \times \frac{x^2 + y^2 - pV^2 t_m^2}{4kt_m} \quad (12)$$

The solution of the simultaneous equations 11 and 12 is:

$$V = \frac{60r}{t_m} \sqrt{\frac{2.303 \log_{10}\left(\frac{v_m}{pv_2}\right) + \frac{p-1}{p}}{2.303 \log_{10}\left(\frac{v_m}{pv_2}\right) + p - 1}} \text{ cm/hr} \quad (13)$$

$$k = \frac{r^2}{240pt_m} \times \frac{(p-1)^2}{2.303 \log_{10}\left(\frac{v_m}{pv_2}\right) + p - 1} \text{ cm}^2/\text{sec}$$

where $t_m$ is in minutes.

Alternatively, the two values of v may be chosen in a particular ratio. If $v_1 = nv_m$ we have:

$$V = \frac{60r}{t_m} \sqrt{\frac{2.303 \log_{10}\left(\frac{t_m}{nt_1}\right) + 1 - \frac{t_m}{t_1}}{2.303 \log_{10}\left(\frac{t_m}{nt_1}\right) - 1 + \frac{t_1}{t_m}}} \text{ cm/hr} \quad (14)$$

$$k = \frac{r^2}{240t_m} \times \frac{\frac{t_m}{t_1} + \frac{t_1}{t_m} - 2}{2.303 \log_{10}\left(\frac{t_m}{nt_1}\right) - 1 + \frac{t_1}{t_m}} \text{ cm}^2/\text{sec}$$

where t is in minutes.

Formulae 13 and 14 are not as accurate as 6 and 10 as the point of maximum temperature rise cannot be determined very accurately, especially at slow sap speeds.

II. A Method for Slow Rates of Flow: If it is desired to distinguish accurately between rather slow rates of flow (V less than 10 or 15 cm/hr) and definitely zero flow, a very sensitive system is obtained by recording the temperature at two points at equal distances above and below the heater. If $v_1$ and $v_2$ are now the temperature rises at time t at $(x_1, y_1)$ and $(x_2, y_2)$ respectively, then:

$$4kt \ln\left(\frac{v_1}{v_2}\right) = (x_2^2 + y_2^2) - (x_1^2 + y_1^2) + 2Vt(x_1 - x_2) \quad (15)$$

If now $x_2 = -x_1$ and $y_2 = -y_1$, we have:

$$V = \frac{k}{x_1} \ln\left(\frac{v_1}{v_2}\right) \text{ cm/sec}$$

Thus, if r is the distance between the heater and each temperature measuring probe, and $\theta$ is the angle between the direction of flow and the line of probes and heater,

$$V \cos\theta = \frac{8289k}{r} \log_{10}\left(\frac{v_1}{v_2}\right) \text{ cm/hr} \quad (16)$$

In this case the measurement gives the component of the sap speed along the heater probe direction, whereas the previous formulae for a single probe, in which x and y occur only in the form $x^2 + y^2 = r^2$, give the magnitude of V but no precise information about the direction of flow. As the probe moves further from the x-axis the effect is simply to reduce the v-scale of the (v, t) curve without altering its shape. This is shown by putting $x_1^2 + y_1^2 = x_2^2 + y_2^2$ in equation 15; the resulting equation does not contain t, showing that at any instant the temperatures at two points equidistant from the heater are always in the same ratio.

Equation 16 is useful for ratios of $v_1$ to $v_2$ up to about 20, corresponding to $V \cos\theta = 18.0$ cm/hr

when $r = 1.5$ cm and $k = 0.0025$ cm$^2$/sec. To use this system the apparatus was modified to record either $v_1$ (as usual) or the difference, $v_1 - v_2$. As $v_1$ is recorded the output is occasionally switched to $(v_1 - v_2)$, thus checking that the ratio $v_1/v_2$ is constant with time. The value of k is found using the previous formulae, and used in equation 16 to obtain V cos $\theta$.

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# IDENTIFICATION AND ESTIMATION OF CATABOLIC PATHWAYS OF GLUCOSE IN FRUITS[1,2]


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The catabolism of glucose and its breakdown products in plants has recently undergone intensive studies in many laboratories (1). Glucose is believed to be utilized for respiratory functions mainly by way of the Embden-Meyerhof-Parnas (EMP) glycolysis and to some extent via the pentose phosphate pathway (2, 6). On the other hand, information on the overall catabolism of glucose in fruit is rather limited although the operation of the tricarboxylic acid cycle (TCA) process, which presumably plays an important role in the catabolism of acetate and pyruvate, has been demonstrated in tomato and pepper (4, 7, 10). In the present work, studies have been extended to include the identification and estimation of pathway participations in glucose catabolism of intact fruits. Using the radiorespirometric method of Wang et al (11), the occurrence of an oxidative pathway involving a preferential oxidation of C-1 of glucose was detected in four varieties of fruits tested. Quantitative evaluation of pathway participations in green mature tomatoes revealed that as much as 84 % of the catabolized glucose was routed via the EMP glycolytic pathway in conjunction with the TCA processes.

## Materials and Methods

Fruits: The tomato, cucumber, lime and orange fruits used in the present experiments were obtained from the local market. Specimens for each experiment were selected on the basis of uniformity in size and degree of maturity. In the case of tomato experiments mature fruit (grown to full size) of the Michigan variety furnished by the Field and Bagley greenhouse, Salem, Oregon, were picked immediately prior to the experiments.

Carbon-14 Labeled Substrates: Glucose-1, -2, and -6-$C^{14}$ were obtained from the National Bureau of Standards through the kind cooperation of Dr. H. S. Isbell. Glucose-U-$C^{14}$ was purchased from Tracerlab Inc. Glucose-3(4)-$C^{14}$ was prepared in this laboratory according to the method of Wood et al and was reported to be labeled almost exclusively (97 to 98 %) on carbon atom 3 and 4 (12).

Administration of Labeled Substrate: Aqueous solution of labeled glucose was introduced into the fruit by one of two methods. In the qualitative survey of the occurrence of the direct oxidative pathway in various fruits, a cylindrical well (4.5 mm diameter and 25 mm in depth) was bored into the center of the test fruits, into which was introduced 0.2 ml of the solution containing 0.50 mg of labeled glucose having a radioactivity of 0.5 to 1.0 $\mu$c. The well was then sealed with the aid of paraffin. Inasmuch as the area of the well was defined, it is reasonable to believe that in each variety of fruit tested, the labeled substrate was exposed to a constant area of fruit tissue. Test experiments designed for evaluation of the validity of this method of administering the substrate revealed that results obtained with fruits of a given variety were reproducible within 15 % and is, therefore, considered to be satisfactory for qualitative studies.

In the case of quantitative estimation of pathways in tomatoes the substrate solution was introduced into fruit, selected to have weights very close to each other, by means of a modified vacuum infiltration


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technique. The stem was removed from each of the tomatoes immediately prior to the experiment. One tenth of a milliliter of the solutions containing 2.08 mg (0.50 $\mu$c) of the specifically labeled glucose was delivered onto the stem scar of the fruit under a partial vacuum. Upon restoration of atmospheric pressure, the substrate solution was rapidly drawn into the fruit. The procedure was repeated once again except 0.2 ml of water was used as flushing agent. It had been established by preliminary experiments with tomatoes that the labeled substrate was evenly distributed in the vascular tissue of the fruit, and excellent reproducibility (within 5 %) was observed among replicate experiments with respect to the radiochemical recovery in the respired $CO_2$.

TIME COURSE EXPERIMENTS: The utilization of individually labeled substrates in fruit with respect to its conversion to respired $CO_2$ was examined in the following manner (11). The fruit, after the administration of the specifically labeled glucose, was placed in a dark respiration chamber equipped with an inlet tube for the sweeping gas (air, oxygen or nitrogen) and an outlet tube leading to a NaOH trap for the collection of respired $CO_2$. The flow rate of the sweeping gas was measured by a master flowmeter connected to individual chambers by means of a bypass manifold equipped with three-way stopcocks. The NaOH trap consisted of a sintered glass sparger immersed in 25 ml of 0.5 *N* $CO_2$-free NaOH. Periodically, this solution was replaced and the trapped $CO_2$ was converted to $BaCO_3$ for radioactivity assay by the addition of a $BaCl_2$-$NH_4Cl$ solution.

UTILIZATION OF GLUCOSE-U-$C^{14}$ BY TOMATO: In order to trace the overall fate of the labeled glucose in tomatoes, 20 $\mu$c of glucose-U-$C^{14}$ was introduced into a tomato fruit weighing 94 g by the vacuum infiltration method. The time course of the combustion of glucose was followed by the assay of the $C^{14}$ in the respired $CO_2$. The experiment was terminated after 9 hours, at which time the radiochemical recovery of the substrate activity in the respired $CO_2$ had declined to a relatively lower rate. The fruit was rapidly homogenized in a Waring blendor and the homogenate extracted exhaustively with 90 % alcohol in a Soxhlet extractor. The alcohol extract was evaporated in vacuo to a syrup and the latter was taken up in water and extracted exhaustively with petroleum ether to remove fatty material. The defatted aqueous solution was then subjected to the successive operation of Dowex-50 and Dowex-3 ion exchange resin columns to remove ionizable compounds such as amino acids and fruit acids. The effluent of the resin column was then processed for the radioactivity assay of the carbohydrate materials of the fruit by means of the wet combustion method (5).

RADIOACTIVITY ASSAY METHOD: The samples of respiratory $CO_2$ in the form of $BaCO_3$ were mounted on aluminum planchets by the centrifugation technique and counted with a thin-window Geiger-Müller tube. Organic materials were assayed for their respective radioactivity by the prior conversion to $BaCO_3$ according to the conventional wet-combustion method (5). All necessary corrections were applied in the conventional manner and all countings were carried out to a standard deviation of 2 %.

## RESULTS AND DISCUSSION

DETECTION OF THE ALTERNATE PATHWAY: In a series of preliminary experiments designed to detect the occurrence of a direct oxidative pathway in tomato fruit, glucose-1-$C^{14}$ and glucose-6-$C^{14}$ were introduced separately by the "well method" into four varieties of fruit. The radiochemical recovery of the substrate activity in the respiratory $CO_2$ was followed for as long as 80 hours. The observed ratios of Gl-1/Gl-6 (average of duplicate experiments), representing the ratios of percent radiochemical recovery of $C^{14}O_2$ from fruits metabolizing glucose-1-$C^{14}$ vs. that from glucose-6-$C^{14}$, are given in table I.

TABLE I

COMBUSTION OF GLUCOSE-1-$C^{14}$ AND GLUCOSE-6-$C^{14}$ BY FRUITS IN AIR

| FRUIT | $C^{14}O_2$ FROM GLUCOSE-1-$C^{14}$/$C^{14}O_2$ FROM GLUCOSE-6-$C^{14}$ | | | |
|---|---|---|---|---|
| | 20 HRS | 30 HRS | 45 HRS | 80 HRS |
| Tomato | 2.3 | 1.9 | 1.5 | 1.5 |
| Cucumber | 3.9 | 3.1 | 2.6 | ... |
| Lime | 2.3 | 2.0 | 1.9 | ... |
| Orange | 1.9 | 1.8 | 1.7 | 1.9 |

Since combustion of the carbon atoms of glucose by way of the EMP and TCA processes results in equal rates of conversion of C-1 and C-6 of glucose to $CO_2$, the preferential conversion of C-1 to $CO_2$ observed in every variety of fruit tested, therefore, suggests the operation of an oxidative pathway involving a $C_1$—$C_5$ cleavage. The latter is also supported by the finding that the ratios of Gl-1/Gl-6 decrease with time in every case. In order to elucidate further the nature of the direct oxidative pathway in question, similar experiments were carried out in atmospheres of air, oxygen or nitrogen for a period of 96 hours using tomatoes as the test system. The average Gl-1/Gl-6 ratio obtained in three identical experiments are given in table II.

In the case of the experiments carried out in the oxygen atmosphere, one finds the Gl-1/Gl-6 ratio is considerably lower when compared to that observed in the air experiment, although the overall radiochemical recovery in the respired $CO_2$ from fruits metabolizing either glucose-1-$C^{14}$ or glucose-6-$C^{14}$ were higher in the oxygen experiment. This is understandable in view of the fact that the TCA process is generally considered to be an aerobic mechanism. Under nitrogen atmosphere, the radiochemical recovery in $C^{14}O_2$ from the labeled glucose was considerably lower, although it is interesting to note that the ratio Gl-1/Gl-6 increased with time in contrast to

TABLE II

COMBUSTION OF GLUCOSE-1-$C^{14}$ AND GLUCOSE-6-$C^{14}$ BY TOMATO FRUITS UNDER VARIOUS ATMOSPHERES

| ATMOSPHERE | % RADIOCHEMICAL RECOVERY IN $C^{14}O_2$ | | | |
|---|---|---|---|---|
| | 20 HRS | 30 HRS | 45 HRS | 96 HRS |
| AIR | | | | |
| Glucose-1-$C^{14}$ | 6.9 | 9.8 | 12.1 | 17.1 |
| Glucose-6-$C^{14}$ | 2.8 | 4.4 | 7.4 | 11.6 |
| Ratio $\frac{Gl\text{-}1}{Gl\text{-}6}$ | 2.5 | 2.2 | 1.6 | 1.5 |
| OXYGEN | | | | |
| Glucose-1-$C^{14}$ | 8.1 | 12.5 | 16.2 | 22.7 |
| Glucose-6-$C^{14}$ | 5.0 | 7.6 | 10.2 | 16.9 |
| Ratio $\frac{Gl\text{-}1}{Gl\text{-}6}$ | 1.6 | 1.6 | 1.6 | 1.4 |
| NITROGEN | | | | |
| Glucose-1-$C^{14}$ | 3.8 | 6.6 | 9.6 | 13.6 |
| Glucose-6-$C^{14}$ | 4.1 | 5.9 | 7.1 | 8.9 |
| Ratio $\frac{Gl\text{-}1}{Gl\text{-}6}$ | 0.9 | 1.1 | 1.3 | 1.5 |

the findings in the oxygen or air experiments. This finding, primarily as a result of the declining rate of conversion of C-6 to $CO_2$, together with the understanding that phosphogluconate decarboxylation pathway can operate without molecular oxygen (3), led us to believe that the phosphogluconate decarboxylation pathway is probably functioning in tomatoes.

FATE OF THE LABELED GLUCOSE IN FRUIT: In order to estimate the relative contribution of the catabolic pathways of glucose (administered) in tomatoes, it is essential to know the exact amount of administered glucose actually engaged in the catabolic processes and then the fate of individual carbon atoms of glucose in these processes. One can visualize that the labeled glucose once introduced into the fruit is subjected to the following processes, namely; 1) dilution with unlabeled fruit glucose resulting in a gradual decrease of the specific activity of the labeled substrate, 2) catabolic degradation leading to the formation of intermediates and to some extent metabolic $CO_2$, 3) incorporation into polysaccharide molecules resulting in the removal of labeled glucose from immediate catabolic functions. When the interval radiochemical recovery of the $CO_2$ from fruit metabolizing glucose-U-$C^{14}$ is examined on a time course basis one finds that the interval (hourly) recovery increases with time in the early phase reflecting the active catabolism of the labeled glucose. This is soon followed by a steady decline evidently as a result of the gradual dilution of labeled glucose by fruit glucose and the removal of the labeled glucose from the active metabolic pool.

In view of the satisfactory reproducibility observed in the preliminary study of substrate administration, it is reasonable to believe that comparable rates of diffusion of the labeled substrate into the fruit vascular tissue were realized within each series of experiments. The slope and height of the interval radiochemical recovery curves from experiments using specifically labeled glucose samples should then reflect directly the relative rates of conversion of individual carbon atoms of glucose to $CO_2$, hence the metabolic pathways followed by the respective carbon atoms.

In figure 1 is given the time course of the interval radiochemical recoveries from tomatoes metabolizing glucose-1, -2, -3(4), and -6-$C^{14}$, respectively. These results represent the average of several series of experiments. That the bulk of glucose is catabolized by way of EMP glycolytic pathway followed by pyruvate decarboxylation to acetate is indicated by the prompt and profuse recovery of C-3(4) in the respiratory $CO_2$. Acetate formed in this manner is presumably metabolized by way of the TCA process (10, 4), as also supported by the finding that the cumulative recovery of C-2 of glucose (equivalent to acetate carboxyl in the EMP-TCA scheme) in the respiratory $CO_2$ (insert in fig 1) is considerably higher than that of C-6 of glucose (equivalent to acetate methyl) and eventually results in an overall ratio of 2 to 1 for $CO_2$ from C-2 versus $CO_2$ from C-6 of glucose. Similar findings have been reported in studies with proliferating yeast in which the TCA process is considered to be responsible for both respiration and biosynthesis of various carbon skeletons (9). The preferential utilization of acetate methyl carbon (hence C-6 or C-1 of glucose) in biosynthesis is thought to account for the observed ratio of $C_6/C_2$ of glucose in $CO_2$.

The preferential recovery of C-1 in the respiratory $CO_2$ over that of C-6 of glucose is observed throughout the entire duration of the experiment. This fact is in accordance with the occurrence of the phosphogluconate decarboxylation pathway in tomato fruits observed in the qualitative studies.

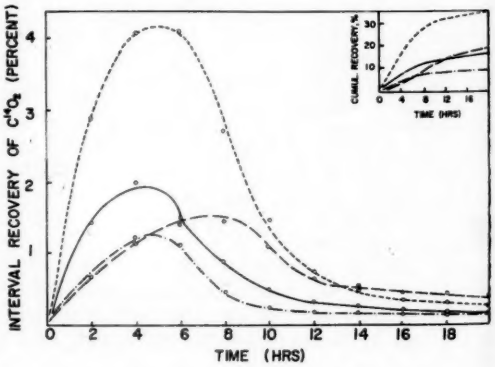


FIG. 1. Interval and cumulative radiochemical recovery in $CO_2$ from tomato fruits metabolizing glucose-1,-2,-3(4), and -6-$C^{14}$ respectively.

Glucose-1-$C^{14}$ ———; glucose-2-$C^{14}$ — — —; glucose-3,4-$C^{14}$ - - - - - - - - -; glucose-6-$C^{14}$ — . — . —.

QUANTITATIVE ESTIMATION OF THE CATABOLIC PATHWAYS OF GLUCOSE: Bearing the foregoing discussion in mind it is then possible to estimate quantitatively the pathway participations in the utilization of the portion of labeled glucose actually catabolized in the fruit making use of the cumulative radiochemical recoveries observed in the glucose experiments. The assumptions required for the derivation of the necessary equations (9) are given as follows: 1) The EMP glycolytic pathway and a direct oxidative process presumably in the nature of phosphogluconate decarboxylation, account for the primary breakdown of glucose. The operation of other minor pathways, although possible, are ignored in the present derivation. 2) The conversion of C-1 of glucose to $CO_2$ in the phosphogluconate decarboxylation is a rapid process. 3) The pentose formed in the phosphogluconate decarboxylation is not engaged extensively in further catabolic processes. 4) The trioses formed in the glycolytic process are equivalent to each other in respect to further metabolic reactions. 5) The pyruvate derived from trioses is decarboxylated promptly giving rise to acetate which is in turn catabolized mainly by way of the TCA processes.

On the basis of these assumptions one may first estimate the net amount of the administered labeled glucose engaged in metabolic functions. For each mole of glucose catabolized by way of glycolysis one would expect one mole of $CO_2$ from *either* C-3 *or* C-4 of glucose, and similarly one mole of $CO_2$ would be recovered from C-1 of glucose catabolized via the direct oxidative pathway. The percent fraction of the labeled glucose catabolized by the fruit can then be estimated according to equation 1:

$$G_t = (G_1 - G_6) + G_{3(4)} \qquad (1)$$

where $G_t$ represents the amount of administered glucose engaged in catabolic functions and $G_1$, $G_6$, and $G_{3(4)}$ are cumulated radiochemical recovery in $CO_2$ from fruits metabolizing equal amounts of the respectively labeled glucose at time t. As shown in the insert of figure 1, the cumulated radiochemical recovery in $CO_2$ from glucose-3(4)-$C^{14}$ levels at approximately 9 hours. If one chooses data at this point for calculation, one finds the amount of labeled glucose catabolized (i.e., labeled glucose molecules undergone metabolic degradation to intermediates and $CO_2$) amounts to 35 % of the administered quantity as calculated according to equation 1. This implies that 65 % of the administered labeled glucose remained in the carbohydrate fraction of the fruit in the form of some simple saccharides or polysaccharides. In a separate time course experiments using glucose-U-$C^{14}$ as tracer, it was found that 61 % of the administered glucose remained in the carbohydrate fraction of the fruit after the experiment was terminated at 9 hours.

Knowing the amount of glucose actually engaged in catabolic functions, it was then possible to determine quantitatively the extent of participation of catabolic pathways in this fruit according to the following equations:

Let $G_e$ be the percent fraction of glucose catabolized via the EMP pathway and $G_p$ be the percent fraction of glucose catabolized via the phosphogluconate decarboxylation pathway. Assuming C-6 of glucose is combusted exclusively by the EMP-TCA pathway, then,

$$G_p = \frac{G_1 - G_6}{G_t} \qquad (2)$$

and

$$G_e = 1 - G_p \qquad (3)$$

It is evident that equations 2 and 3 give a minimum value for $G_p$ and a maximum value for $G_e$, since any recovery of C-6 in $CO_2$ via the recombination of trioses followed by phosphogluconate decarboxylation will tend to reduce the value of $G_p$. In view of the fact that recovery rate of C-6 of glucose in $CO_2$ was moderately high and prompt in the early phase of the glucose experiment and the findings of Seegmiller et al (8), it is reasonable to believe that a portion of C-6 of glucose may have been combusted to $CO_2$ via the direct oxidative pathway, consequently the true value of $G_p$ is probably a little higher than that calculated according to equation 2. The exact nature of the oxidation processes for C-6 to $CO_2$ in tomatoes is currently under investigation in this laboratory.

In the present study with mature tomato fruits it was estimated according to the foregoing equations 2 and 3 that approximately 84 % of the glucose degraded biologically was catabolized by way of the EMP-TCA pathway, the remaining fraction being presumably routed through the phosphogluconate pathway.

## SUMMARY

The operation of an oxidative pathway involving a $C_1$—$C_5$ cleavage for glucose catabolism has been demonstrated in tomatoes, cucumbers, limes and oranges. A method has been devised in estimating pathway participation in tomatoes which reveals that 84 % and 16 % of the fruit glucose is catabolized via the EMP-TCA processes and the alternate oxidative pathway respectively.

The authors wish to express their appreciation to Professor E. Hansen of the Horticulture Department of Oregon State College for his generous help throughout this work.

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# DARK FIXATION OF $CO_2$ BY SUCCULENT LEAVES: CONSERVATION OF THE DARK FIXED $CO_2$ UNDER DIURNAL CONDITIONS[1,2]

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The characteristic ability of the succulents to incorporate a large net amount of $CO_2$ in the form of organic acids has been recognized for a long time (9). Although the biochemical mechanisms of this phenomenon have received a great deal of attention, the physiological role has not been fully understood. In the course of our studies on the pathways of the dark fixation of $CO_2$ (6, 7), it was observed that the $C^{14}O_2$ incorporated in the dark was not lost in the subsequent light period but appeared to be utilized directly in the photosynthesis. From these and other considerations we are impressed with a possibility that the special features of Crassulacean acid metabolism have an adaptive advantage.

## Materials and Methods

A random sample of young *Bryophyllum calycinum* Salisb. leaves (approximately 2 to 3 cm long, wet weight 0.5 to 0.6 g), picked from the apex of the plant was placed in the apparatus described in a previous communication (6) and permitted to fix $C^{14}O_2$ (generated from 4.6 mg of $BaC^{14}O_3$, specific activity 120 c/mg) for 30 minutes in the dark. Two of the leaves were homogenized immediately with boiling 80 % ethanol and an aliquot counted for determination of total activity. All samples were counted at infinite thinness using a Micromil gas flow counter. The total homogenate was then filtered through Whatman no. 1 paper, washed three times with small portions of boiling 80 % ethanol, and an aliquot of the total filtrate analyzed by paper chromatography as indicated below. The remaining leaves were removed, placed on moist filter paper, and illuminated with a photoflood lamp (Photospot R.S.P. 2, G.E. 115 to 120 v) placed 31 to 32 inches away. Cool air circulated over the surface of the leaves with a fan to prevent over-heating. At no time did the temperature at the surface of the leaves exceed 28° C. At various intervals pairs of leaves were removed and homogenized in boiling 80 % ethanol. The determination of the total activity in the homogenate and the chromatographic analysis of the filtrate was carried out as described below. As a control, one pair of leaves was exposed 30 minutes to $C^{14}O_2$ in the dark, flushed free of $C^{14}O_2$, kept in the dark for 120 minutes, and homogenized in boiling 80 % ethanol.

Identification of the labeled photosynthetic and dark products was made on aliquots of the 80 % ethanol soluble fractions. This was accomplished by two dimensional chromatography on Whatman no. 1 filter paper using phenol (80) : water (20) (w/w) in the 1st direction and *n*-butanol (74) : acetic acid (19) : water (50) (v/v/v) in the 2nd. Radioautographs were made with "no-screen" x-ray film. In all experiments, non-labeled glucose and sucrose were co-chromatographed with the extract. Identification of the radioactive compounds was made by superposition. Activity was determined directly on the paper with an end-window Geiger tube.

An alternate technique was employed to ascertain whether the $C^{14}O_2$ fixed in the dark was released to

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[2] This investigation was supported by a research grant from the Tobacco Industry Research Committee. The facilities of the Allan Hancock Foundation were generously provided.

the atmosphere during the subsequent light period. Matched pairs of leaves were exposed to $C^{14}O_2$ in the dark for 60 minutes. As a control, one pair was immediately homogenized in boiling 80 % ethanol, and the radioactivity determined on an aliquot. The other pair of leaves was placed in a glass chamber and illuminated with three 120-watt incandescent lamps placed 24 inches from the surface of the leaves. During the illumination, moist air was flushed through the chamber and the $CO_2$ was trapped in 1 *N* NaOH, precipitated as $BaCO_3$, and counted with a gas flow Geiger tube.

Experiments were designed to test the effect of previous conditions of light or dark on the subsequent photosynthetic $CO_2$ incorporation. Intact plants were placed either in a completely dark room for 17 to 24 hours or in direct sunlight. At the beginning of the experiment matched pairs of leaves were removed, placed in the glass illumination chamber, exposed to $C^{14}O_2$, and allowed to photosynthesize for 30 minutes. The leaves were then homogenized in boiling 80 % ethanol and the total activity measured on an aliquot as described above.

## Results and Discussion

The total amount of radioactivity retained by the leaves at each time interval of illumination after the dark incorporation of $C^{14}O_2$ is presented in table I. These data represent the averages of three experiments. A most interesting finding is that the total amount of radiocarbon incorporated in the dark period remained constant during the subsequent light period as well as during the dark control.

In our previous research concerning the dark metabolism of $C^{14}O_2$ in Bryophyllum (7) we demonstrated that no detectable activity was incorporated into either the carbohydrates or their phosphorylated derivatives, all activity being associated with the organic and amino acids. Chromatographic analysis of the extract from the dark control confirmed this. However, when the leaves were transferred to light in the absence of exogenous $C^{14}O_2$ significant activity was associated with the sucrose from the 80 % ethanol soluble fraction. Figure 1 presents the radioautographs from the dark and light extracts. It is clear that upon exposure of the leaf to light, not only was there a conservation of the $C^{14}O_2$ fixed in the dark, but that $CO_2$ was utilized in the formation of photosynthetic products.

Table I

Radioactivity Retained by Bryophyllum Leaves after Illumination

| Duration of light | 0 | 10 | 30 | 60 | 120 | (120) Dark control |
|---|---|---|---|---|---|---|
| Total activity in homogenate * (cpm/mg leaf) | 737 | 781 | 706 | 750 | 813 | 745 |

* Samples were counted within a statistical error of 5 %.

Table II

Loss of Dark Fixed $C^{14}O_2$ During the Subsequent Light Period

| Experiment | Total activity fixed in dark (cpm) | Total free $C^{14}O_2$ trapped as $BaCO_3$ (cpm) | Percent total activity lost |
|---|---|---|---|
| I | $2.4 \times 10^5$ | $5.4 \times 10^3$ | 2.2 |
| II | $5.0 \times 10^5$ | $9.1 \times 10^3$ | 1.8 |

Evidence for this conservation of dark-fixed $CO_2$ during photosynthesis was obtained by the 2nd technique in which the $CO_2$ was trapped during the process of photosynthesis. The data from typical experiments are shown in table II. It is clear that less than 3 % of the total activity accumulated by the leaves was in equilibrium with the external atmosphere. This experiment indicates that the $CO_2$ incorporated during the dark was efficiently retained, and utilized during subsequent photosynthesis in the leaf. Similar results have been observed with a marine flagellate, *Dunaliella euchlora* by Ryther (5). Ryther interprets his data as evidence for a preferential utilization of endogenous $CO_2$.

The ability of succulents to incorporate large amounts of $CO_2$ in the dark, and subsequently utilize this $CO_2$ for photosynthesis is a possible biochemical adaptation to arid conditions. The thick spongy leaves and stems with rather impermeable cuticles are useful anatomical structures for water conservation. Since photosynthetic activity takes place at those times when water loss is maximal, re-utilization by the leaf of the dark incorporated $CO_2$ would tend to minimize the need for opening the stomata to permit gas exchange, and might aid in the conservation of water. Physiological observations of Loftfield (2) support this hypothesis. He demonstrated that, unlike non-succulents, the stomata of the succulents remained closed during the day, but opened at night to permit gas exchange.

Thomas et al (10) have shown that under normal atmospheric $CO_2$ concentration (0.03 %), light deacidification takes place in succulent leaves. However, when the leaves are exposed to high concentrations of $CO_2$ (5 to 10 %), this light deacidification can be retarded, and under certain conditions light acidification can be induced. The only known mechanism for the synthesis of carbohydrates from dicarboxylic acids involves decarboxylation and reversal of glycolysis. The observations of Thomas et al (10) could be interpreted as evidence for the preferential carboxylation of ribulose diphosphate as compared to phosphoenol-pyruvate. During the light period following dark acidification $CO_2$ liberated via decarboxylation immediately enters into the light $CO_2$ fixation mechanism. However, when the ribulose diphosphate pathway for $CO_2$ fixation is saturated at high $CO_2$ concentrations in the light, the acidification system involving phosphoenol-pyruvate carboxylase can be utilized.

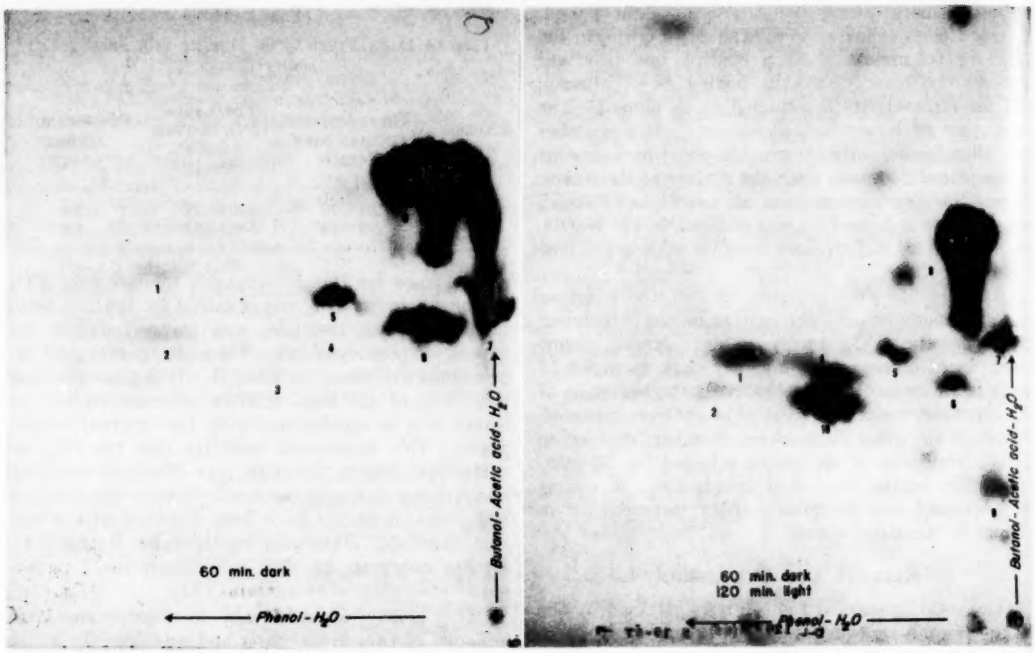


FIG. 1. Radioautograms of two dimensional paper chromatograms of extracts from 60-minute dark fixation of $C^{14}O_2$, and 60-minute dark fixation of $C^{14}O_2$ followed by 120-minute photosynthesis in absence of $C^{14}O_2$. Compounds indicated are: 1. alanine, 2. glutamine, 3. asparagine, 4. glycine-serine, 5. glutamate, 6. aspartate, 7. citrate-isocitrate, 8. malate, 9. phosphoglycerate, and 10. sucrose.

The effects of previous exposure of the leaves to light or dark on the total photosynthetic $CO_2$ fixation are presented in table III. The experiments were designed so that the leaves of the plants pretreated in the light would be depleted of most of their organic acids. These data are very similar to those presented by Sen and Leopold (8). There are several interpretations that can be made from these results. Sen and Leopold favor the view that there is some temporary impairment of the mechanisms of photosynthesis during the previous exposure to dark. This impairment was manifested in the diminution of the $C^{14}O_2$ incorporated. Another interpretation is possible if one postulates that the rates of photosynthesis subsequent to either pretreatment are approximately the same. The $CO_2$ fixed during the dark periods would provide a considerable store of endogenous $CO_2$ which was preferentially utlized. This would result in the decreased utilization of the exogenous $CO_2$. It is well known from the work of Pucher et al (3, 4) that there is an inverse quantitative relationship between total titratable acids and net carbohydrate stores in succulent leaves subjected to diurnal conditions.

TABLE III

PHOTOSYNTHETIC $C^{14}O_2$ FIXATION LEAVES PRETREATED IN DARK OR IN LIGHT

| PRETREATED IN LIGHT | | PRETREATED IN DARK | | |
|---|---|---|---|---|
| HOURS OF PRE-TREATMENT | TOTAL CPM | HOURS OF PRE-TREATMENT | TOTAL CPM | RATIO LIGHT/DARK |
| 8 | $38.2 \times 10^5$ | 17 | $7.7 \times 10^5$ | 5 |
| 8 | $23.9 \times 10^6$ | 24 | $1.9 \times 10^6$ | 12 |

The known pathways for the interconversion of Krebs cycle organic acids and carbohydrates depend upon decarboxylation and the reversal of glycolysis. The labeling experiments of Varner and Burrell (11) and Gibbs (1) completely support this hypothesis. This evidence in conjunction with the experiments presented in this paper indicates that the $CO_2$ derived from decarboxylation of the organic acids is retained by the cell and metabolized via the normal photosynthetic mechanisms.

## SUMMARY

Detached leaves of *Bryophyllum calycinum* are able to fix, in the dark, large amounts of $C^{14}O_2$ in the form of organic and amino acids. When these leaves are exposed to light in the absence of $C^{14}O_2$, radioactivity is found in the carbohydrate fraction. The dark fixed $C^{14}O_2$ is efficiently conserved and metabolized during subsequent photosynthetic reactions.

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# RESPIRATION OF DEVELOPING ROSE PETALS[1]


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Some of the earliest investigations on the relation of respiration to development were carried out on flowers and floral structures. The Araceae were found particularly suitable for this purpose because of the high metabolic rates of such structures as the spadix and appendix. Respiratory activity in the aroids was found by van Herk (1, 2, 3) and later by James and Beevers (4) to exhibit a sudden rise followed by a decline. The maximum rate of respiration occurred when the appendix or spadix had reached its maximal size.

The purpose of this study was to examine the over-all gas exchange and some specific metabolic reactions in relation to growth and senescence of rose petals. The energy for expansion and unfolding of the petals is no doubt supplied by respiration. Attempts were made, therefore, to investigate the relation between the respiratory and the energy processes during the course of development of rose petals.

## Materials and Methods

*Rosa hybrida*, cultivar Better Times grown in a commercial greenhouse was used in all these experiments. The flowers were picked each morning and only the five outermost petals were used for respiration measurements.

Gas exchange measurements were made in the Warburg apparatus at 25° C with a shaking rate of 112 cycles per minute and a 3-cm amplitude. The results are reported as $Q_{O_2}$, $Q_{O_2}(N)$, and $Q_{O_2}(P)$, which designate oxygen uptake in cubic millimeters per hour per mg dry weight, mg nitrogen, or mg phosphorus, respectively, and $Q_{CO_2}$, which designates carbon dioxide production in cubic millimeters per hour per mg dry weight. The rose petals from 8 flowers were randomized, cut into 12-mm$^2$ pieces with scissors, and five pieces which varied in dry weight from 15 to 30 mg were placed in Warburg vessels with 3 ml of 0.05 M phosphate or McIlvaines buffer. Penetration of substrates or inhibitors was aided by vacuum infiltration (5). Preliminary experiments showed that the infiltration procedure had no effect on the respiration rate. Homogenate preparation is described in the text.

The photoreversibility of carbon monoxide was examined by irradiating with a no. 2 Mazda photoflood lamp immersed in the bath 10 cm from the side of the Warburg flask. Cyanide inhibition was measured using the center well solutions of Robbie (6) and Laties (7). 2,4-dinitrophenol (DNP) of the appropriate concentrations was prepared in 0.067 M phosphate buffer and then adjusted to pH 4.3. The other inhibitors were prepared in 0.05 M phosphate buffer and then adjusted to pH 4.5. Insoluble phosphorus was determined by the method of Krotkov and Helson (8) and nitrogen was determined by the semi-


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[3] Deceased.


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micro Kjeldahl method. All values reported are means of at least duplicate determinations with a maximum variation of 8 % between samples.

## Results

About 250 rose buds of uniform size were selected on the plants so that we could follow changes in respiration of the petals as they developed. At about 8 A.M. each day a sample of flowers was picked. Sampling began when the diameter of the flower buds reached 15 mm. The initial respiration rates were measured daily; the drift in respiration in relation to unfolding is presented by figure 1 and a diagram of the stages of development of the rose petal is shown in figure 2. Each stage in this figure represents about two days. The stage of slow petal expansion (fig 2, A and B) is characterized by a continual decline in $Q_{O_2}$ to a minimum value. The stage of rapid petal expansion (fig 2, C) is characterized by a sudden increase in $Q_{O_2}$. The stage of the full petal expansion (fig 2, D through F) shows a slow decline in the $Q_{O_2}$ from a maximal value.

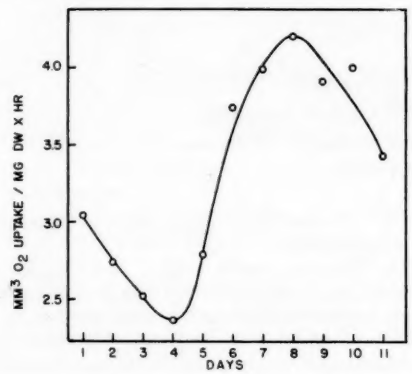



Fig. 1 (*above*). Drift of the initial oxygen uptake of pieces from the 5 outer rose petals with development based on dry weight.

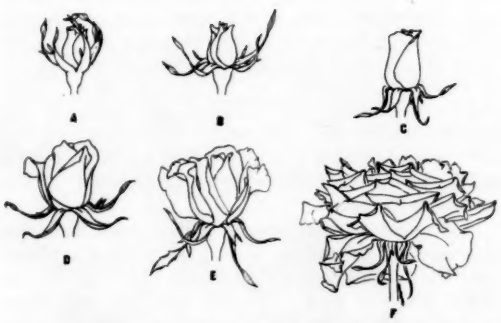
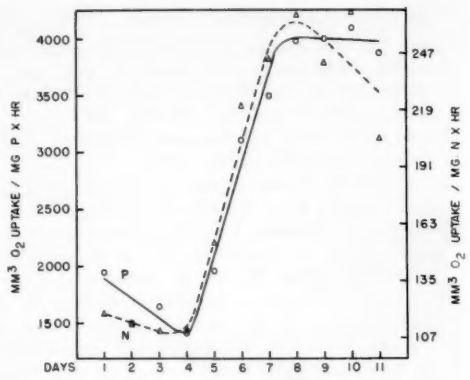



Fig. 2 (*below*). Development of the entire rose flower during the period when respiration measurements were made. The stages shown are about 2 days apart (see text for details).



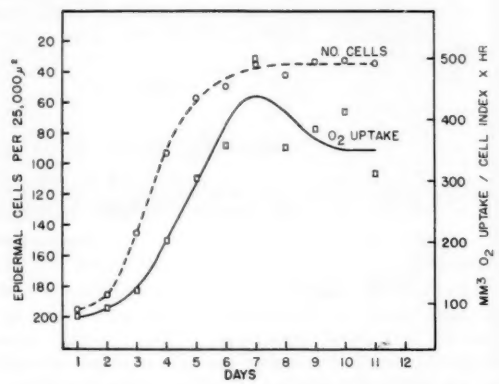
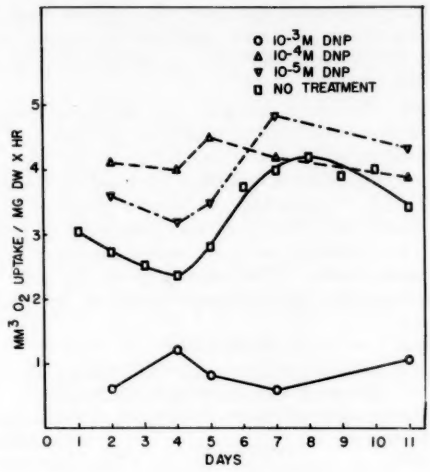




Fig. 3 (*above*). Drift of the initial oxygen uptake of pieces from the 5 outer rose petals with development based on total nitrogen (N) and insoluble phosphorus (P).

Fig. 4 (*below*). Drift of the initial oxygen uptake of pieces from the 5 outer rose petals with development based on a cell index. The decreasing number of epidermal cells per 25,000 $\mu^2$ with development was used to determine the cell index.

The drift in respiration was also examined using total nitrogen and insoluble phosphorus as the base for oxygen uptake. This represents an attempt to base respiratory measurements on an active fraction of the cell constituents. Respiration calculated on a nitrogen or insoluble phosphorus basis has a similar pattern (fig 3) to that shown by respiration based on dry weight (fig 1).

The petal area used for each respiration measurement was approximately constant. A count of the number of upper epidermal cells per unit area provided a means of expressing respiration on a cellular basis, on the assumption that the ratio of epidermal to mesophyll cells remains constant throughout growth



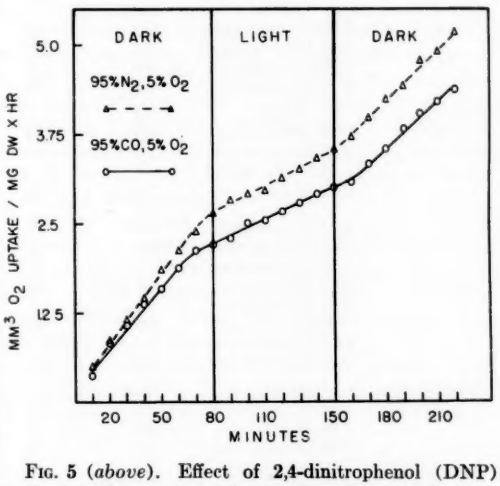




FIG. 5 (*above*). Effect of 2,4-dinitrophenol (DNP) on the initial oxygen uptake of pieces from the 5 outermost rose petals with development.

FIG. 6 (*below*). Oxygen uptake of pieces from the 5 outer rose petals during slow petal expansion in the presence of carbon monoxide (95 % CO + 5 % $O_2$) and in its absence (95 % $N_2$ + 5 % $O_2$).

due to cell expansion. No cell divisions were observed during petal expansion. The number of cells in a unit area decreased as the petals increased in size. The oxygen uptake on a cell index basis (fig 4) resembled the pattern of the respiratory drift based on dry weight, nitrogen and insoluble phosphorus. This was calculated by dividing the oxygen uptake of the petal area used by the total number of upper epidermal cells normalized by dividing by the number of upper epidermal cells of the first day.

The nature of the metabolic changes associated with development was examined in experiments with 2,4-dinitrophenol (DNP). It was shown above that the respiration of rose petals varied with their stage of development. It was of interest, therefore, to see whether the rate of oxygen uptake by this material could be stimulated by DNP and whether the degree of stimulation is related to the stage of development. The respiratory rates of rose petal pieces subjected to different concentrations of DNP are plotted against time in figure 5. The curves are compared with a normal respiratory curve of developing rose petals. The stimulatory effect of DNP at $10^{-5}$ M and $10^{-4}$ M was more pronounced during the stage of slow petal expansion (fig 2, A and B) than during the stage of full expansion (fig 2, D through F). The importance of selecting the proper DNP concentration for demonstrating stimulation of oxidative activity has been shown for several tissues (9). In the case of avocado fruit slices, for example, it was found (10) that $10^{-4}$ M DNP was most effective in bringing about an increase in oxygen uptake, while $10^{-3}$ M DNP was definitely inhibitory. With the rose petals, the greatest percentage of increase of oxidative activity was brought about by $10^{-4}$ DNP and took place during the period preceding full petal expansion.

A comparison of the metabolism of the rose petals was attempted at the stages of slow and full petal expansion. The respiratory quotient was found to be 1.0 at both stages. Increasing the oxygen concentration from 20.9 % (air) to 100 % had no effect on respiration. Evidently the terminal oxidase system was saturated with respect to oxygen at 20.9 %. Data of table I indicate that during the slow petal expansion stage (fig 1, A and B) the petals had a lower rate of anaerobic $CO_2$ production than during the fully expanded stage (fig 1, D through F). Fluoride at 0.03 M inhibited the anaerobic $CO_2$ production of petals moderately in the stage of slow petal expansion and strongly during the fully expanded stage (table I).

Characterization of the terminal oxidase of the petals was attempted by the use of the heavy metal inhibitors azide, cyanide, and carbon monoxide. At the lower concentration, cyanide and azide produced a slight stimulation of oxygen uptake in the slowly expanding petals (table II). They were moderately to severely inhibitory at the higher concentration. The fully expanded petals were strongly inhibited at

TABLE I

EFFECT OF FLUORIDE ON THE ANAEROBIC $CO_2$ PRODUCTION OF ROSE-PETAL PIECES

| STAGE OF PETAL EXPANSION AND INHIBITOR | $Q_{CO_2}$ | % INHIBITION |
|---|---|---|
| Slow (fig 1, A and B) | | |
| None | 0.77 | ... |
| 0.03 M NaF | 0.47 | 39 |
| Full (fig 1, D–F) | | |
| None | 2.1 | ... |
| 0.03 M NaF | 0 | 100 |

TABLE II

EFFECT OF CYANIDE AND AZIDE ON THE OXYGEN UPTAKE OF ROSE-PETAL PIECES

| STAGE OF PETAL EXPANSION AND INHIBITOR | CONCENTRATION | $Q_{O_2}$ | % INHIBITION |
|---|---|---|---|
| Slow (fig 1, A and B) | | | |
| None | ..... | 2.4 | .. |
| KCN | $1\times10^{-4}$ M | 2.7 | −13 |
| KCN | $5\times10^{-3}$ M | 0.6 | 75 |
| None | ..... | 2.3 | .. |
| $NaN_3$ | $1.7\times10^{-4}$ M | 3.1 | −35 |
| $NaN_3$ | $1.7\times10^{-3}$ M | 1.8 | 22 |
| Full (fig 1, D–F) | | | |
| None | ..... | 4.1 | .. |
| KCN | $1\times10^{-4}$ M | 1.3 | 68 |
| KCN | $5\times10^{-3}$ M | 0.12 | 97 |
| None | ..... | 3.4 | .. |
| $NaN_3$ | $1.7\times10^{-4}$ M | 0.55 | 84 |
| $NaN_3$ | $1.7\times10^{-3}$ M | 0.01 | 100 |

the lower concentration of cyanide and azide and completely inhibited at the higher concentrations. The oxygen uptake of the slowly expanding petals was inhibited only about 10 % in the dark by carbon monoxide (95 % CO + 5 % $O_2$) compared to the oxygen uptake of similar petals in 95 % $N_2$ + 5 % $O_2$ (fig 6). Irradiation of the flasks caused a decrease in oxygen uptake compared with the dark period. The inhibition in light compared with dark was about 55 %. The effect of light on decreasing oxygen uptake was not due to carbon monoxide since the control flasks responded similarly. The oxygen uptake of fully expanded petals was inhibited by carbon monoxide in the dark and this inhibition was partially reversed by light (fig 7).

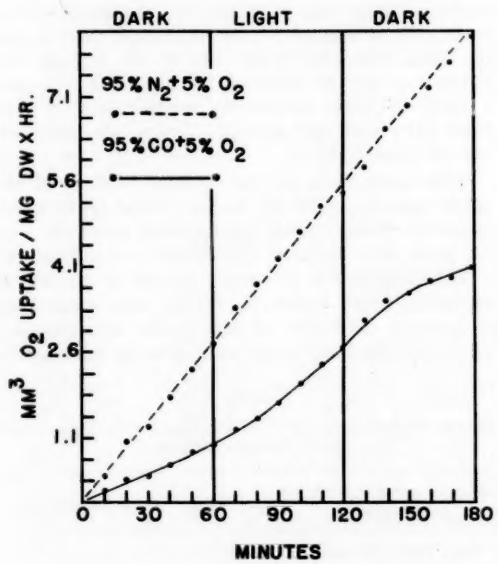



FIG. 7. Oxygen uptake of pieces from the 1st 5 outer fully-expanded rose petals in the presence of carbon monoxide (95 % CO + 5 % $O_2$) and in its absence (95 % $N_2$ + 5 % $O_2$).

The influence of malonate on the initial respiration of rose petals was examined at the slow and full petal expansion stages. The petals in the slow expansion stage were only weakly inhibited by malonate (table III). Increasing the concentration of malonate caused an increase in the percentage of inhibition, but inhibition at high concentrations is probably non-specific. The respiration of the fully expanded petals was markedly inhibited by 0.05 M malonate (table III) but not by 0.01 M malonate. The inhibition of respiration caused by 0.05 M malonate was not reversed by pyruvate or fumarate.

TABLE III

EFFECT OF SOME ORGANIC ACIDS AND GLUCOSE ON THE OXYGEN UPTAKE OF ROSE-PETAL PIECES

| % INHIBITION | TREATMENT | $Q_{O_2}$ | STAGE OF PETAL EXPANSION |
|---|---|---|---|
| Slow (fig 1, A and B) | None | 2.7 | .. |
| | 0.05 M malonate | 2.3 | 15 |
| | None | 3.3 | .. |
| | 0.04 M malonate | 2.9 | 12 |
| | 0.06 M malonate | 2.3 | 30 |
| | 0.08 M malonate | 2.0 | 39 |
| | 0.10 M malonate | 1.8 | 45 |
| Full (fig 1, D–F) | None | 3.2 | .. |
| | 0.05 M malonate | 1.2 | 62 |
| | 0.03 M pyruvate | 3.7 | −16 |
| | 0.05 M malonate + 0.03 M pyruvate | 1.4 | 56 |
| | None | 3.3 | .. |
| | 0.05 M malonate | 1.0 | 70 |
| | 0.05 M fumarate | 2.9 | 12 |
| | 0.05 M malonate + 0.05 M fumarate | 0.7 | 79 |
| | 0.01 M malonate | 3.2 | 3 |
| | 0.03 M pyruvate | 3.9 | −18 |
| | 0.01 M malonate + 0.03 M pyruvate | 3.2 | 3 |
| Slow (fig 1, A and B) | None | 3.4 | .. |
| | 0.03 M pyruvate | 3.2 | 6 |
| | 0.05 M glucose | 3.4 | 0 |
| | 0.06 M succinate | 3.7 | − 9 |
| Full (fig 1, D–F) | None | 3.1 | .. |
| | 0.03 M pyruvate | 3.9 | −26 |
| | 0.05 M glucose | 3.7 | −19 |
| | 0.06 M succinate | 3.6 | −16 |

Several substrates were infiltrated into rose petals at the slow and full petal expansion stages. During the slow petal expansion stage, no substrate tested elicited any extra respiration (table III). Fully expanded petals had a slightly increased respiration after infiltration of several substrates. Starvation of the excised petals by holding for 24 or 48 hours at 25° C did not change their response to the added substrate.

A comparison of the oxygen uptake of a homogenate from rose petals and rose-petal pieces was made. Homogenates were prepared by grinding 5 g

of rose petals with 25 ml of 0.10 M phosphate buffer pH 7.4 in a chilled mortar with sand. The endogenous oxygen uptake of homogenates prepared from rose petals was linear for about 30 minutes and then gradually declined. Cyanide at $5 \times 10^{-3}$ M did not inhibit the oxygen uptake of a homogenate prepared from petals at the stage of the slow petal expansion or at the stage of full petal expansion (table IV). However, oxygen uptake of the petal pieces of either stage was almost completely inhibited at $5 \times 10^{-3}$ M cyanide.

The results of the heavy metal inhibitor studies on fully expanded petals suggested that cytochrome oxidase might be operative as the terminal oxidase. Attempts to assay for cytochrome oxidase with homogenates were not successful. Further attempts to demonstrate cytochrome oxidase in the particulate matter obtained from high-speed centrifugation were not successful.

It is obvious from the experiments with heavy metal inhibitors and DNP that considerable metabolic changes occur during the growth and development of rose petals. In order to carry out further studies on the changes of the enzymatic activities and phosphorylations, attempts were made to prepare an active mitochondrial fraction from rose-petal homogenates. Homogenates of rose petals were made by blending 30 g of tissue for one minute with 60 ml of a solution 0.4 M in sucrose and 0.1 M in phosphate adjusted to pH 7.1. The blendor was operated at 50 volts. The homogenate was centrifuged first at 500 $\times$G for five minutes, and the supernatant solution from this centrifugation was further centrifuged at 17,000 $\times$ G for 15 minutes to obtain the mitochondrial fraction. The mitochondrial fraction so obtained stained with Janus green, but did not catalyze the oxidation of any acids in the tricarboxylic acid cycle. The pH and temperature of the preparation were carefully controlled. Throughout the preparation, the pH was kept between 6.8 and 7.1 and the temperature was between 0° and 3° C. Tonicity of the medium was varied (0.25 to 0.75 M sucrose). However, the mitochondrial fractions were metabolically inactive. Therefore, it appears that inactivity of the preparations cannot be attributed to the factors of pH, temperature, or tonicity in the range examined. The possibility of the release of an inhibiting substance during the preparation of the mitochondria was tested. The rose-petal supernatant solution was added to preparations of avocado and pea mitochondria. The avocado particles were prepared according to the procedure of Biale et al (11), while the pea mitochondria were obtained by a method similar to that of Millerd et al (12) for mung beans. The oxidation of succinate by pea and avocado mitochondria was inhibited by the rose-petal supernatant solution (table V). A similar inhibition was obtained for the oxidation of $\alpha$-ketoglutarate and malate. These results suggest that the damage to the rose-petal mitochondria was irreversible and took place during the course of preparation.

TABLE IV

EFFECT OF CYANIDE ON THE OXYGEN UPTAKE OF ROSE-PETAL PIECES AND HOMOGENATES

| STAGE OF PETAL EXPANSION, INHIBITOR AND TYPE OF MATERIAL | $Q_{O_2}(N)$ | % INHIBITION |
|---|---|---|
| Slow (fig 1, A and B) | | |
| No inhibitor | | |
| Piece | 110 | .. |
| Homogenate | 140 | .. |
| $5 \times 10^{-3}$ M KCN | | |
| Piece | 23 | 79 |
| Homogenate | 130 | 7 |
| Full (fig 1, D–F) | | |
| No inhibitor | | |
| Piece | 250 | .. |
| Homogenate | 70 | .. |
| $5 \times 10^{-3}$ M KCN | | |
| Piece | 10 | 96 |
| Homogenate | 100 | −43 |

TABLE V

EFFECT OF ROSE SUPERNATANT SOLUTION ON THE OXYGEN UPTAKE OF AVOCADO AND PEA MITOCHONDRIA

| COMPONENTS IN REACTION MIXTURE * | OXYGEN UPTAKE IN MM³/VESSEL/HOUR | |
|---|---|---|
| | AVOCADO MITOCHONDRIA | PEA MITOCHONDRIA |
| Mitochondria + succinic acid | 200 | 181 |
| Mitochondria + succinic acid + rose supernatant | 70 | 90 |
| Mitochondria + rose supernatant | 35 | 54 |
| Rose supernatant + succinic acid | 24 | ... |
| Rose supernatant | 23 | 15 |

* Reaction mixture: 60 micromoles of succinic acid, 30 micromoles of phosphate, 3 micromoles of ATP, 12 micromoles of Mg, and 0.5 ml mitochondrial enzyme of avocado or pea as indicated, with or without 1.0 ml of rose supernatant as indicated in 3.0 ml of 0.5 M sucrose solution adjusted to pH 7.1 at 20° C.

## DISCUSSION

The course of respiration in developing rose petals followed a definite pattern resembling that of the climacteric rise in fruits (13). There was a marked rise in respiration during the expansion of the rose petals. In the case of fruits, the respiratory rise, which is associated with the ripening process, takes place normally after the fruits are fully expanded and mature. In the case of developing rose petals, the respiratory rise accompanies development and maturation, characterized morphologically by the rapid expansion and unfolding of the petals. From a morphological viewpoint, the respiratory rise of rose petals

is not comparable to the climacteric rise of fruits. Respiration studies of entire flowers did not reveal a respiratory rise at any time after commercial harvest (14). The centripetal development of the petals in a multipetaled flower probably confounds the respiratory rise observed here in one whorl of petals. Bonner and Honda (15) found no indication of a respiratory rise in petals of mature Camellia flowers already at maximum size.

The stimulatory effect of certain DNP concentrations was found to be at a minimum when the endogenous respiration of the rose petal was at its peak. The respiration of the early stage of growth can effectively be raised by eliminating its dependence on phosphorylation with the aid of DNP. The pronounced DNP effects in the early developing stage of rose petals suggest that the phosphorylative capacity is limiting, perhaps due to relatively low levels of phosphate acceptor. As unfolding progresses the increased synthetic activity might be responsible for a greater turn-over of the acceptor. An analysis for adenylates, however, is needed before any definite conclusions can be drawn.

Along with changes in the respiratory pattern associated with the growth of rose petals, observations were made on changes in the metabolism by inhibitor studies. The results of these inhibitor studies suggest that the terminal oxidase of the full expansion stage of the rose petals is principally a metal enzyme and probably cytochrome oxidase. However, the nature of the terminal oxidase of the slow expansion stage of the rose petals was not ascertained.

The high rate of oxygen consumption by the homogenate was striking. The cyanide insensitivity of these preparations resembles that of the spinach chloroplast preparation of Laties (16). It appears that the homogenization process rendered the cyanide-sensitive system inoperative.

The inhibitions of enolase by fluoride has been amply demonstrated in plants (5, 17). The anaerobic $CO_2$ production of the rose petals was markedly inhibited by fluoride, and this fluoride inhibition is more pronounced at the full expansion stage than at the slow expansion stage. The studies on the effect of malonate also indicate that possible changes have taken place in the aerobic metabolism with the respiratory rise. Pyruvate, succinate, and glucose all increased the oxygen uptake of the rose petals in the full-expansion stage, but they were without any marked effect on the rose petals in the slow-expansion stage.

There was a rise in the anaerobic $CO_2$ production along with the respiratory rise in the developing rose petals. The rate of the anaerobic $CO_2$ production of the petals in the full-expansion stage was 2.7 times as high as the rate of the petals of the slow-expansion stage. Thus, the ratio of the anaerobic $CO_2$ production to the aerobic $CO_2$ production was 0.31 for the petals in the slow-expansion stage and 0.52 for the petals in the full-expansion stage. Apparently, a Pasteur effect occurs only in the full-expansion stage.

In recent years, cytoplasmic particles, believed to be mitochondria, have been isolated from a number of plant tissues. The mitochondria are known to be the site of oxidation of Krebs-cycle acids and are able to carry out phosphorylation coupled with oxidation (18). The failure to obtain metabolically active mitochondrial preparations from the rose petals prevented further enzymatic characterization of the respiratory pattern of rose petals. The supernatant solution from rose petals was shown to be inhibitory to the normal oxidative activities of mitochondria from avocado and pea. This inhibitory effect suggests the possibility that there is an inhibitor present in the cell sap of rose petals, which interferes with the isolation of an active mitochondrial preparation.

## Summary

The respiration of rose petals was studied in relation to growth and development. A definite respiratory pattern was found in the developing rose petals. It consisted of a respiratory rise before the opening of the flower, a respiratory peak when the flower fully opens, and a respiratory drop following the complete opening of the flower.

DNP at concentrations of $10^{-5}$ M and $10^{-4}$ M increased and at $10^{-3}$ M inhibited the respiration of the developing rose petals. The stimulatory effect of DNP was more pronounced during the early stage of petal unfolding than during the stage of full expansion.

Metabolic changes accompanied the growth and development of the rose petals. While the nature of the terminal oxidase from inhibitor studies was not defined during the stage of slow petal expansion, it appeared that cytochrome oxidase participated in the respiration during full-petal-expansion stage. Fluoride was an inhibitor of anaerobic carbon dioxide production and it was more effective during the stage of full petal expansion. Malonate was only a respiratory inhibitor during the full-petal-expansion stage, and the inhibition was not reversible on the addition of fumarate or pyruvate. Pyruvate, succinate and glucose increased the respiration during the full-petal-expansion stage only. The oxygen uptake of the homogenate was cyanide insensitive. Attempts to study metabolic changes in the developing rose by mitochondrial preparation were not successful. Indications were obtained that the supernatant solution from rose petals inhibited the activity of the mitochondria.

The authors are indebted to Dr. F. M. Scott for the cell counts of the rose petals during development.

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# PHOTOCONTROL OF ALCOHOL, ALDEHYDE, AND ANTHOCYANIN PRODUCTION IN APPLE SKIN[1]

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While examining the influence of light on anthocyanin synthesis in pieces of apple skin floating on sucrose solutions, we noticed that light strongly inhibited the formation of the characteristic apple odor (10). In the present work the influence of visible radiation on aldehyde, alcohol, and anthocyanin formation was examined in detail. Radiation was found to suppress oxygen consumption markedly with accompanying sparing of sugar, suggestive of the Pasteur effect. One of the aromatic rings in the anthocyanin molecule is derived from acetate (1, 3, 4, 11), and anthocyanin synthesis in many plants requires radiation. The action spectra suggest that the photoreceptive pigment is the oxidized form of an acyl coenzyme A dehydrogenase (9, 10), in accord with the utilization of acetate. Further evidence that two photoreactions with the same photoreceptor are required for anthocyanin synthesis in apple skin is presented here.

## MATERIALS AND METHODS

Early-harvested Jonathan and Arkansas apples were held at 0° C after being sealed in 1-bushel bags made of 0.38-mm polyethylene. Narrow strips of green areas of the apple skin were removed with a White Mountain apple peeler. The strips were cut into 7- × 7-mm pieces with razor blades mounted in a holder. The pieces, which had some cortical tissue attached, were washed twice with 0.3 M sucrose and then for one hour in running tap water. Fifty-one pieces (25 $cm^2$) of apple skin were placed cuticle side up in a 10-cm Petri dish containing 15 ml of 0.3 M sucrose. The Petri dishes were held in darkness or irradiated with a cool white fluorescent source of radiant energy, giving a maximum illumination of about 2000 foot-candles (ft-c).

All inhibitors and substrates used were dissolved in 0.3 M sucrose and the final solution was adjusted to pH 4. All temperatures reported were measured in the sucrose solution in which the pieces of apple skin floated.

In all experiments in which anthocyanin synthesis was examined, the Petri dishes containing the apple-skin pieces were held 24 hours in darkness after irradiation at a temperature three degrees lower than that during the irradiation period.

The anthocyanin content of the pieces of apple skin was determined on an extract made at 1° C in

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darkness from 25 pieces (12 $cm^2$) of apple skin in 10 ml of 1 % HCl in methanol. The absorbance measurements were converted to moles anthocyanin/$cm^2$ by the use of a molar-extinction coefficient ($\epsilon$) of $3.43 \times 10^4$ at 530 $m\mu$. The molar-extinction coefficient was determined on an authentic sample of cyanidin 3-galactoside (idaein).

The aldehyde and the alcohol production of the pieces of apple skin were determined enzymatically on a steam distillate of apple-skin pieces. All the pieces of apple skin and the sucrose solution from one Petri dish were quantitatively transferred to a 50-ml flask and steam-distilled until 25 ml of distillate was collected. The distillate was stored in sealed vials at 1° C until analyzed. The aldehyde content of the distillate was assayed enzymatically by a slight modification of the procedure of Racker (7). The following were placed in a 1-cm light-path pyrex-glass cuvette: 0.05 ml of 1 M potassium phosphate buffer, pH 7.2; 0.1 ml of $3 \times 10^{-3}$ M DPNH (reduced diphosphopyridine nucleotide) in distilled water; and 2.75 ml of distillate containing aldehyde or distilled water. An initial optical-density reading was taken at 340 $m\mu$ (maximum for DPNH) and then 0.05 ml of a 1 % solution of crystalline yeast alcohol dehydrogenase (Worthington) in 0.1 % bovine serum albumin and 0.01 M potassium phosphate, pH 7.2, were added and the cuvette contents stirred. The reduction of the aldehyde was completed in about two minutes, as indicated by the constancy of optical-density readings. The final density reading was subtracted from the initial density reading and the aldehyde concentration was calculated by use of a conversion factor of 0.1 micromole aldehyde equals a density change of 0.21 at 340 $m\mu$.

The alcohol content of the distillate was assayed by a modification of the enzymatic procedure of Bonnichsen and Theorell (2). Yeast alcohol dehydrogenase was found to be just as satisfactory as liver alcohol dehydrogenase for this assay at the levels of alcohol measured. The following were placed in a 1-cm light-path pyrex-glass cuvette: 0.1 ml of $1 \times 10^{-2}$ M DPN (diphosphopyridine nucleotide) in distilled water; 1.0 ml or less of distillate containing alcohol; and a buffer-semicarbazide solution to make a total volume of 3.2 ml. The buffer-semicarbazide solution had the following composition: 3 vol. of 0.1 *N* NaOH + 7 vol. of 0.1 *N* glycine in 0.1 *N* NaCl + 1 vol. of 0.1 *N* semicarbazide hydrochloride in 0.1 *N* NaOH. An initial optical-density reading was taken at 340 $m\mu$ and then 0.05 ml of the yeast alcohol dehydrogenase enzyme was added and the cuvette contents stirred. The final optical-density reading was made after exactly 30 minutes at room temperature. Standard curves were determined for each series of analyses by use of the following amounts of alcohol: 0, 0.10, 0.20, 0.40, and 0.60 micromole. The initial optical-density reading was subtracted from the final value minus the blank, and the alcohol concentration was determined from the standard curve.

The alcohol dehydrogenase enzyme will react with several aldehydes, ethanol and higher aliphatic alcohols. Therefore, the values were expressed in this paper only as alcohol and aldehyde since the enzyme is not exclusively specific for acetaldehyde and ethyl alcohol. However, according to White (12), acetaldehyde accounts for 60 % of the carbonyl compounds and ethyl alcohol for 77 % of the alcohols in apple-juice volatiles.

Respiration measurements were made by standard Warburg manometric procedures with shaking at 80 cycles/minute. The 0.3 M sucrose in which the pieces of apple skin were placed was about 7 mm deep in the manometric vessel. During shaking in light some of the pieces had the cuticle side down or were obscured by others floating over them. Carbon dioxide assimilation was measured in Warburg vessels at 2 mm $pCO_2$ and a $C^{14}O_2$ activity of 30 $\mu$C/vessel.

## Results

The time courses of alcohol and aldehyde formation in pieces of Arkansas apple skin in darkness or light were determined at several temperatures. Syntheses of these compounds were markedly influenced by both light and temperature (fig 1 a and 1 b).

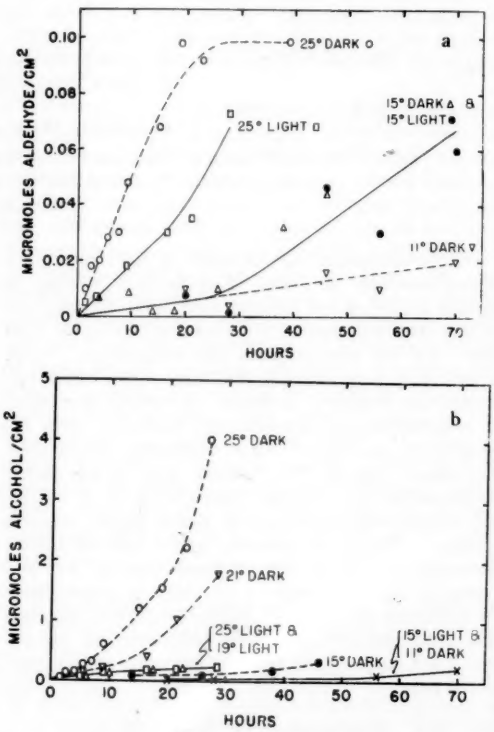


Fig. 1. Dependence of aldehyde and alcohol formation in pieces of skin of Arkansas apple floating on 0.3 M sucrose on the time of holding in darkness or light at the indicated temperatures: (a) aldehyde; (b) alcohol.

TABLE I

ANTHOCYANIN FORMATION IN PIECES OF ARKANSAS APPLE SKIN AFTER A 24-HOUR IRRADIATION PERIOD AT 21° C IN THE PRESENCE OF SEVERAL COMPOUNDS

| COMPOUND | $3 \times 10^{-1}$ | MOLES ANTHOCYANIN $\times 10^{-8}$/CM² FORMED AT INDICATED CONCENTRATION | | | | |
|---|---|---|---|---|---|---|
| | | $10^{-1}$ M | $10^{-2}$ M | $10^{-3}$ M | $10^{-4}$ M | $10^{-5}$ M |
| Pyruvate | ... | 0.85 * | 1.45 | 1.27 | 1.52 | ... |
| Shikimate | ... | ... | ... | 1.24 | ... | ... |
| Acetate | ... | ... | ... | 0.99 * | 1.20 | 1.52 |
| Iodoacetate | ... | ... | ... | 0 * | ... | ... |
| Sucrose | 1.24 | ... | ... | ... | ... | ... |

* Injury evident.

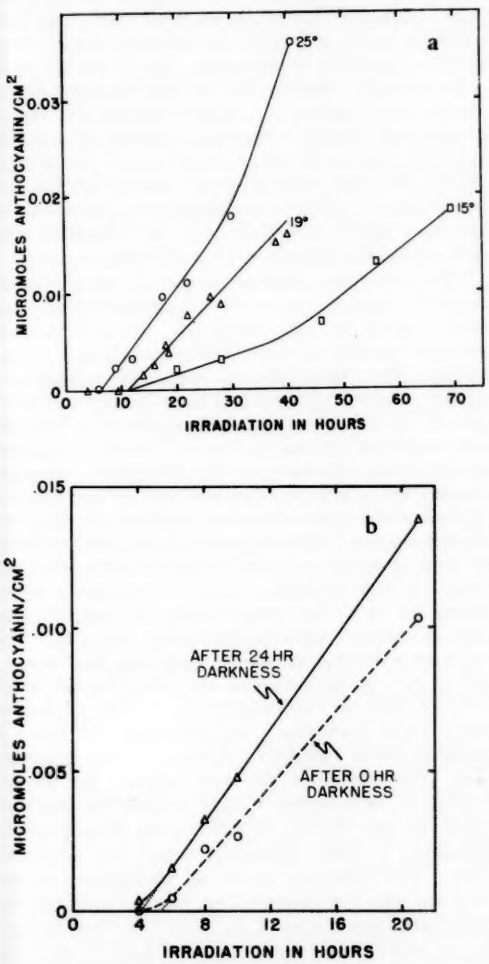


FIG. 2. Dependence of anthocyanin formation in pieces of apple skin floating on 0.3 M sucrose on the time of irradiation under a fluorescent light source: (a) pieces of Arkansas apple skin at the indicated temperatures; (b) pieces of Jonathan apple skin at 25° C with and without prior holding on the sucrose solution in darkness.

The time course of anthocyanin formation in pieces of Arkansas apple skin irradiated at several temperatures was examined. The rate of anthocyanin formation and the induction period before anthocyanin formation begins are both affected by temperature (fig 2 a). The time course of anthocyanin synthesis in pieces of skin of Jonathan apple held in the dark for 24 hours before irradiation was compared with that in pieces of apple skin irradiated immediately after preparation (fig 2 b). The temperature during this experiment was 25° C. There is considerable formation of apple volatile substances during the dark period. The standard errors for the anthocyanin contents after 21 hours of irradiation were $0.08 \times 10^{-8}$ and $0.07 \times 10^{-8}$ moles anthocyanin/cm² for samples held in darkness and not held there, respectively. The corresponding standard errors after 8 hours of irradiation were 0.012 and $0.004 \times 10^{-8}$ moles anthocyanin/cm².

Possibly stimulatory or inhibitory effects of several substances on anthocyanin synthesis were examined. Jonathan apple skin pieces formed 0.99, 0.88, 0.81, and $0.88 \times 10^{-8}$ moles anthocyanin/cm², respectively, in 0, 0.014, 0.028, and 0.056 M ethanol after a 24-hour irradiation period at 25° C, with a standard error of $0.12 \times 10^{-8}$ moles anthocyanin/cm². Anthocyanin production in pieces of Arkansas apple skin after a 24-hour irradiation period at 21° C in the presence of various compounds is shown in table I. Injury, seen as discoloration of the skin pieces, was evident in the $10^{-1}$ M pyruvate and $10^{-3}$ M acetate solution. Phosphate at $10^{-3}$ M did not influence aldehyde or alcohol production.

The fresh weight of the Arkansas apple skin tissue was 0.084 g/cm². This weight includes a considerable amount of attached cortex tissue. The cortical tissue held at 21° C in darkness and at 25° C in light did not produce significant amounts of aldehyde in 24 hours. The alcohol production of the cortical tissue is about 10 % that of the skin tissue.

Anthocyanin formation is first evident at the edges of the pieces. This is obviously a wound reaction not eliminated by washing.

The respiration, measured as $O_2$ uptake and $CO_2$ production, of pieces of Arkansas apple skin held on 0.3 M sucrose for 24 hours at pH 4 was as follows:

| | $O_2$ Uptake moles $\times 10^{-8}/cm^2/hr$ | $CO_2$ Production moles $\times 10^{-8}/cm^2/hr$ |
|---|---|---|
| Light | 16.0 | 19.0 |
| Dark | 26.5 | 32.3 |

The ratios of $CO_2$ production to $O_2$ uptake (R.Q.) were 1.19 and 1.22 in light and dark, respectively. Apparent respiration of freshly prepared pieces of green Arkansas apple skin floating on 0.3 M sucrose was reduced to zero by 1000 ft-c illumination. The extent to which this apparent suppression of respiration was a result of photosynthesis was not determined. The photosynthetic rate of the irradiated pieces was about one-tenth that of a young soybean leaf as measured by $C^{14}O_2$ uptake. An apparent zero respiration on irradiation would imply a very effective recapture of respiratory $CO_2$ by photosynthesis despite the presence of 20 % KOH in the center well of the Warburg vessel and low illumination of some pieces.

## Discussion

Radiation is strikingly effective in controlling metabolism in the apple skin. It is necessary for anthocyanin synthesis and strongly inhibits alcohol formation. Oxygen consumption is also possibly inhibited in light and sugar is spared. The effects are clean-cut for alcohol and anthocyanin syntheses.

Inhibition of alcohol formation by radiation is most evident at 25° C but is still seen at 21° and 15°. Alcohol, anthocyanin, and aldehyde production, $O_2$ uptake, and $CO_2$ production per hour during the first 20 hours at 25° C in 1 $cm^2$ of Arkansas apple skin were as follows:

| | Dark moles $\times 10^{-8}$/ $cm^2$/hr | Light moles $\times 10^{-8}$/ $cm^2$/hr |
|---|---|---|
| Alcohol | 8.5 | 1.0 |
| Aldehyde | 0.5 | 0.2 |
| Anthocyanin | 0.0 | 0.8 |
| $O_2$ (uptake) | 26.5 | 16.0 (apparent) |
| $CO_2$ (production) | 32.3 | 19.0 (apparent) |

The alcohol production, calculated from the difference between $O_2$ uptake and $CO_2$ production in the dark, would be 5.8 moles $\times 10^{-8}/cm^2/hr$ in comparison with the observed value of 8.5 moles $\times 10^{-8}/cm^2/hr$. The discrepancy is very likely due to the markedly different conditions for measurement of the two values. Either value, though, indicates that approximately equal quantities of sugar are fermented and oxidized in darkness.

The alcohol production of the pieces of apple skin in light is reduced to 10 % of the value in darkness or to 15 % if aldehyde is included. The apparent oxygen uptake is reduced about one-half, but it is probably confounded with evolution of oxygen in photosynthesis. A similar suppression of apparent respiration was noted by Siegelman et al (8) for immature rose petals, but possible confounding with photosynthesis would have to be carefully considered.

Aldehyde is an intermediate in alcohol formation. In darkness it presumably would be produced at a steady-state rate depending upon its rate of formation from pyruvate and its reduction to alcohol. The fact that aldehyde does not markedly accumulate in light indicates that the suppression of alcohol formation is controlled at or prior to pyruvate utilization.

The formation of anthocyanin, $C_{15}H_{11}O_6$ for cyanidin, from sugar would not require $CO_2$ evolution or oxygen consumption. The synthesis of 0.8 moles $\times 10^{-8}$ anthocyanin/$cm^2$/hr in apple skin would require as much sugar as 4 moles $\times 10^{-8}/cm^2/hr$ of alcohol formation in darkness. The pieces of apple skin actually produce 8.5 moles $\times 10^{-8}/cm^2/hr$ of alcohol in darkness. A material balance for anthocyanin and alcohol indicates a sparing of sugar in light, similar to the effect of oxygen in the Pasteur effect. The way by which sugar is spared in the Pasteur effect is unknown. The proposed explanations are discussed by Laties (6) and the difficulties with their acceptance are given by Krebs (5).

The tentative conclusions which can be drawn from the time courses of the appearances of the several products are now considered. Time is required for each product to reach a steady-state rate of formation after the apple skin pieces are placed in a sucrose solution. This is less than 0.5 hour at 25° C for aldehyde (fig 1 a) in harmony with a probable high turnover rate for pyruvate. The rate of alcohol accumulation (fig 1 b), on the other hand, increases steadily as would be expected for an end-product dependent on prior aldehyde production. Anthocyanin formation has an induction period with a lag of at least 6 hours at 25° C. About one-fourth (1.5 hours) of this induction period is eliminated by allowing the apple skin pieces to float on a sucrose solution in darkness prior to irradiation (fig 2 b). The 1.5-hour reduction in the lag period is the time required for the formation of the 1st substrate, which requires light for conversion to a 1st light product of anthocyanin formation. An additional 4.5 hours is required before radiation induces anthocyanin formation. This period is the time required for the 1st product of light action to start forming the substrate for a 2nd light action. A steady state of anthocyanin formation is then attained in about one additional hour. The schematic course of anthocyanin formation can now be conceived as follows, with the approximate time required at 25° C for the reaction shown above the arrow to approach steady-state conditions or to reach essential completion:

Sucrose —(30 minutes)→ pyruvate —(1 hour)→ active acetate —(radiation-limited step number 1)→ first product of radiation —(4 hours)→ intermediate reactions including "head to

tail" active acetate condensation product of radiation $\xrightarrow{\text{radiation-limited step number 2}}$ second product of radiation $\xrightarrow[\text{8 hours}]{}$ dark reactions leading to eventual cyclization of a polyacetate compound into the phloroglucinol ring of anthocyanin. The active acetate compound involved in the 1st photoreaction reaches a steady-state condition limited by the utilization of active acetate in respiration through the tricarboxylic acid cycle. That the same photoacceptor is involved in both photoreactions was indicated in the previous work on the action spectrum (10). The time courses of anthocyanin synthesis in seedlings of turnip and red cabbage (9) support these deductions. Turnip seedlings grown in the presence of chloramphenicol have already reached the steady-state formation of the product of the 3rd reaction in darkness. The turnip seedlings show no evidence of the 1st two reactions of the scheme. Red cabbage seedlings, after the photomorphogenic condition is established, have an immediately linear time course of anthocyanin synthesis. The 1st five steps are already at steady state in red cabbage seedlings and only the 2nd photoreaction appears. The red cabbage seedlings can thermally accomplish the 1st photoreaction and also the 2nd, as shown by the appreciable synthesis of anthocyanin in darkness.

## Summary

The time courses of aldehyde, alcohol, and anthocyanin syntheses in light and darkness were measured in apple skins floating on sucrose solutions. Radiation suppresses aldehyde and alcohol syntheses with a sparing of sugar in a manner equivalent to the suppression of fermentation by oxygen and sugar use in fermentative systems. The time course of anthocyanin synthesis indicates that two radiation-limited steps are involved in anthocyanin synthesis in apple tissue.

We are indebted to C. E. Hagen for the manometric measurements.

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# LIPID SOLUBLE PIGMENTS OF WHEAT PLANTS AS RELATED TO HESSIAN FLY INFESTATION[1,2]

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The dark blue-green color of the center leaves of certain wheat plants is the visual means whereby experienced entomologists select hessian fly infested wheat or barley plants. This color is abnormal because center leaves are younger than outer ones and when uninfested are light green in color. In normal plants, the older, outer leaves are darker green than the younger center leaves. Differences in type or concentration of plant pigments may explain these differences.

The objective of this study was to investigate the possible relationship of pigmentation in hessian fly

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infested wheat plants to the concentration of the lipid soluble pigments chlorophyll, carotene, and xanthophyll. It was also desired to determine if any relationship existed between the concentration of these pigments and hessian fly resistance of several varieties.

## Materials and Methods

Uninfested oats and durum (P.I. 94587), Pawnee × 94587 (C.I. 12855), Ponca, Pawnee, Tenmarq and Rival wheat plants were grown in hydroponic solution in the greenhouse (2). Lots of comparable infested and uninfested (normal) Tenmarq and Rival wheat plants were grown in hydroponic tanks as well as in ground beds in the greenhouse.

Center and outer leaves of the plants were harvested when the plants were about 30 days old. Five-gram samples of the fresh leaves were placed in plastic bags and frozen immediately at −20° C. Moisture was determined by drying additional samples at 130° C for five hours.

Analysis for Chlorophyll: The method for chlorophyll determination was that from the Official Methods of Analysis of the A.O.A.C. (1), using the 5-g frozen samples.

Analysis for Carotene and Xanthophyll: Aliquots (100 ml) of the acetone extracts (1) from the chlorophyll determinations were transferred to Skellysolve B, and carotene and xanthophyll were determined by methods which were essentially those described by Gugliemelli and Mitchell (3) for the determination of xanthophyll. A 30-ml portion of a freshly prepared 20 % solution of potassium hydroxide in absolute ethanol was shaken with the Skellysolve B solution for three minutes to saponify completely the chlorophyll. Water was added and the resulting two phases were separated. The alcohol-water phase was extracted twice with 50 ml portions of Skellysolve B. The Skellysolve B solutions were combined, washed thoroughly with water, concentrated to about 30 ml on a steam plate, and transferred quantitatively to a 25 × 70 mm column of a lime-Supercel mixture (3 : 1 by weight). The carotene was eluted with Skellysolve B, and xanthophyll with 5 % absolute ethanol in Skellysolve B (3). The absorbancies of the carotene and xanthophyll solutions were measured at 436 mμ and 444 mμ, respectively, using a Beckman DU Spectrophotometer. Absorptivities for carotene and xanthophyll used for calculating the amount of pigment per g of leaf tissue were 196 and 226, respectively.

## Results and Discussion

The amounts of chlorophyll, carotene and xanthophyll in 6 uninfested wheat varieties and oats grown in hydroponic solution in the greenhouse under comparable conditions are shown in table I. The highest order of resistance is indicated by the number 1, and progressively lower orders of resistance by the numbers 2, 3 and 4. Susceptibility is indicated by the letter S. Oats are generally recognized as being immune to hessian fly attack. On the basis of these data, it was concluded that the quantities of chlorophyll, carotene, and xanthophyll in uninfested wheat leaf tissue bear no relation to the degree of resistance to hession fly attack. When the data were expressed on a dry weight basis, a similar conclusion was reached. These results of quantitative pigment analysis are within the ranges reported by Strain (4) for leaf ma-

Table I

Chlorophyll, Carotene and Xanthophyll Concentrations in Inner and Outer Leaves of Uninfested Oats and Several Uninfested Wheat Varieties Representing a Wide Range of Resistance to Hessian Fly

| Degree of resistance | Variety | Leaf tissue | Dry weight | Chlorophyll | | | Carotene | Xanthophyll |
|---|---|---|---|---|---|---|---|---|
| | | | | a | b | a/b | | |
| | | | % | mg/100 g fresh wt | | | | |
| 1 | Oats | Inner | 12.7 | 82 | 31 | 2.62 | 7.3 | 19 |
| | Oats | Outer | 13.4 | 101 | 42 | 2.42 | 10.0 | 19 |
| 1 | 94587 | Inner | 11.7 | 76 | 29 | 2.58 | 5.9 | 16 |
| | 94587 | Outer | 13.3 | 107 | 45 | 2.41 | 9.0 | 22 |
| 1 | 12855 | Inner | 12.9 | 80 | 30 | 2.67 | 5.9 | 15 |
| | 12855 | Outer | 15.9 | 127 | 51 | 2.47 | 10.8 | 25 |
| 3 | Ponca | Inner | 11.8 | 73 | 29 | 2.53 | 6.3 | 17 |
| | Ponca | Outer | 13.6 | 102 | 43 | 2.39 | 9.4 | 23 |
| 4 | Pawnee | Inner | 12.3 | 77 | 31 | 2.44 | 6.5 | 17 |
| | Pawnee | Outer | 14.1 | 115 | 48 | 2.38 | 10.7 | 25 |
| S | Tenmarq | Inner | 13.2 | 78 | 29 | 2.67 | 6.3 | 18 |
| | Tenmarq | Outer | 15.1 | 120 | 50 | 2.39 | 11.0 | 27 |
| S | Rival | Inner | 13.0 | 78 | 29 | 2.75 | 7.1 | 19 |
| | Rival | Outer | 14.1 | 107 | 44 | 2.42 | 10.5 | 25 |
| | Average | Inner | 12.5 | 77.7 | 29.7 | 2.61 | 6.5 | 17.3 |
| | Average | Outer | 14.2 | 111.3 | 46.1 | 2.41 | 10.2 | 23.7 |

TABLE II

FRESH AND DRY WEIGHTS OF FRACTIONS FROM FIFTY INFESTED AND FIFTY UNINFESTED TENMARCQ WHEAT PLANTS

| LEAF TISSUE | FRESH WEIGHT | DRY WEIGHT |
|---|---|---|
| | *g* | *g* |
| Infested center | 5.3 | 1.1 |
| Infested outer | 8.1 | 1.5 |
| Infested stems | 5.4 | 0.9 |
| Total | 18.8 | 3.5 |
| Noninfested center | 10.2 | 1.8 |
| Noninfested outer | 24.3 | 3.9 |
| Noninfested stems | 18.3 | 2.5 |
| Total | 52.8 | 8.2 |

terial of different plant species. The ratio of chlorophyll a to b was greater in center than in outer leaves.

The effect of infestation on the wet and dry weight of fractions from 50 Tenmarq wheat plants is shown in table II. These data are for plants infested 12 days and harvested 33 days after planting. They demonstrate that the wet and dry weights are considerably less for infested than for the same number of uninfested plants. Similar data were obtained for Rival wheat plants. These data suggest that the hessian fly larvae interfere with the normal metabolism and stunt the growth of the wheat plants.

The concentrations of lipid soluble pigments in the center and outer leaves of infested and uninfested wheat plants are shown in table III. The center and outer leaves of infested plants contained, in general, greater quantities per unit weight of chlorophyll, carotene and xanthophyll than the corresponding leaves of the uninfested plants. The center leaves of the infested plants generally contained more of all three pigments than the outer leaves of the same plants. The converse was true of uninfested plants. When the data were expressed on a dry weight basis, similar conclusions were reached. The ratios of chlorophyll a to b were higher for uninfested plants. These data may explain the visual observation that the center leaves of infested plants are dark blue-green while similar leaves from uninfested plants are much lighter green in color.

In the present work, anthocyanins were not investigated and it is not known whether their production is altered by hessian fly infestation. The investigation of the anthocyanins and other flavanoid pigments in wheat plants is being pursued.

## SUMMARY

Center leaves of hessian fly infested wheat plants contained greater percentages of chlorophyll, carotene and xanthophyll than either the outer leaves of infested plants or comparable leaves of uninfested plants. The center (younger) leaves of uninfested plants contained less of these pigments than the outer leaves of the same plants. The ratios of chlorophyll a to b in both inner and outer leaves were higher for uninfested plants than for infested plants. The unnatural dark blue-green color of the center leaves of hessian fly infested wheat plants may be due to the higher concentrations of chlorophyll a and b. There

TABLE III

CHLOROPHYLL, CAROTENE AND XANTHOPHYLL CONCENTRATIONS IN INNER AND OUTER LEAVES OF INFESTED AND UNINFESTED WHEAT PLANT LEAF TISSUE

| VARIETY | LEAF TISSUE | DRY WEIGHT | CHLOROPHYLL | | | CAROTENE | XANTHOPHYLL |
|---|---|---|---|---|---|---|---|
| | | | a | b | a/b | | |
| | | % | *mg/100 g fresh wt* | | | | |
| Tenmarq * | Uninfested, center | 12.5 | 108 | 43 | 2.52 | 9.1 | 17.5 |
| Tenmarq * | Uninfested, outer | 13.6 | 121 | 53 | 2.31 | 11.3 | 20.7 |
| Tenmarq * | Infested, center | 16.0 | 173 | 79 | 2.20 | 17.0 | 28.9 |
| Tenmarq * | Infested, outer | 16.6 | 156 | 70 | 2.25 | 15.1 | 25.4 |
| Tenmarq ** | Uninfested, center | 10.3 | 52 | 19 | 2.81 | 3.7 | 8.9 |
| Tenmarq ** | Uninfested, outer | 10.4 | 79 | 33 | 2.38 | 5.8 | 11.7 |
| Tenmarq ** | Infested, center | 13.6 | 105 | 45 | 2.36 | 8.2 | 9.5 |
| Tenmarq ** | Infested, outer | 11.6 | 84 | 38 | 2.20 | 6.4 | 9.8 |
| Rival * | Uninfested, center | 14.9 | 108 | 43 | 2.49 | 9.4 | 22.4 |
| Rival * | Uninfested, outer | 14.3 | 105 | 43 | 2.44 | 9.3 | 20.8 |
| Rival * | Infested, center | 16.4 | 161 | 68 | 2.36 | 15.4 | 25.6 |
| Rival * | Infested, outer | 16.2 | 143 | 62 | 2.31 | 13.3 | 23.5 |
| Rival ** | Uninfested, center | 11.8 | 76 | 27 | 2.81 | 6.5 | 16.4 |
| Rival ** | Uninfested, outer | 12.5 | 108 | 45 | 2.41 | 9.8 | 22.7 |
| Rival ** | Infested, center | 13.6 | 116 | 48 | 2.44 | 10.8 | 22.1 |
| Rival ** | Infested, outer | 12.0 | 110 | 46 | 2.37 | 9.6 | 20.2 |

* Grown in ground beds.
** Grown in hydroponic solution.

was no apparent relation between concentration of any of these pigments and resistance among wheat varieties to hessian fly attack.

The authors wish to thank Mr. Robert M. Grossman and Dr. Robert J. Robinson for technical assistance.

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# EFFECT OF OZONE AND OZONATED 1-HEXENE ON RESPIRATION AND PHOTOSYNTHESIS OF LEAVES[1,2]

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Damage to plants from pollutants such as those present in polluted Los Angeles air may be manifested in at least two primary ways: 1st, as visible oxidant damage to the leaves of susceptible plants, where it is apparent that leaf cells have been killed (4, 5); and 2nd, as a decrease in plant growth not accompanied by visible injury (3, 6, 9).

Injury to herbaceous plants from toxicants present in the air of the Los Angeles area can be reproduced by exposing susceptible plants to a mixture of 1-hexene and ozone gas (2). Most previous investigations of the effect of oxidant-type air pollutants have employed this artificial mixture, which can be prepared in the laboratory. The present work was undertaken to investigate the possibility that the rates of photosynthesis or respiration of leaves might be changed by exposure to these gases at concentrations below those required to produce visible damage. Erickson and Wedding (1) found that the photosynthesis of Lemna plants was reduced after exposure to either ozone or ozonated hexene. The respiration rate increased after exposure to the latter gas. Since the exposures used were such that the plants were visibly injured, the reduction in photosynthesis may have been due to the death of many cells. Todd (8) has shown that the respiration of lemon fruit exposed either to ozone alone or to ozonated hexene is greatly increased over the normal, even though the fruit is not visibly injured.

The infrared carbon dioxide analyzer makes possible the measurement of photosynthesis and respiration of intact leaves as affected by these toxic substances. These physiological processes can be followed before, during, and after fumigation of the leaves with the toxic gases.

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## Materials and Methods

For most tests the two primary leaves of bean plants of the Pinto variety were used as the experimental material. The plants were usually 14 days old, and the primary leaves were fully expanded. For tests on citrus leaves, Valencia orange cuttings grown in 1-gallon cans were used. All plants were grown in a greenhouse where the air supplied to the house was filtered through activated charcoal so that toxic oxidants contained in naturally polluted air were removed .

The leaves were enclosed in modified Petri dishes (1.6 × 8.2 cm) having a slit to accommodate the leaf petiole, and with side arms such that 8-mm Tygon tubing could be attached for the incoming and outgoing air streams. The dishes were supported on small wooden adjustable platforms. After the leaf had been inserted, the dish was clamped onto a flat glass plate and held in place with a C-clamp. The leaves were sealed into the containers with modeling clay without detaching them from the plant. For the duration of the experiments the leaves showed no visible changes as a result of the manipulations.

The plants were placed in a light-tight box 26 inches long, 14 inches wide, and 20 inches deep. Six fluorescent lights (20 watt—3 daylight and 3 standard cool white) were spaced across the top of the box. The box was supplied with room air by a small blower. The room was maintained at 20° ± 0.5° C, although the temperature of the box during respiration was approximately 23° C, and when the lights were turned on the temperature rose to approximately 33° C.

The air supplied to the plants was drawn by a diaphragm pump through activated charcoal to remove naturally occurring oxidants. The flow was regulated by two sets of capillaries, one for photosynthesis and the other for respiration. A time clock

operated the lights and the solenoid valves that determined which flow would be used. After the air passed over the leaves it was dried and subsequently entered the carbon dioxide analyzer. The air from each leaf chamber was sampled for 12 minutes, and as many as 8 different samples could be obtained by an arrangement of solenoid valves. Thus a sample was obtained from each leaf approximately every 1.5 hours. If longer or more frequent samples were desired, individual leaves were sampled manually.

A Liston-Becker Carbon Dioxide Analyzer (Model 15) was used in all of these studies, with a range of approximately 150 to 450 ppm $CO_2$. The instrument was calibrated by mixing a known carbon dioxide supply (0.95 % $CO_2$ in $N_2$) with pure nitrogen and calculating the $CO_2$ concentration from the pressure drop across a calibrated capillary. Since the rate of airflow and the dry weights of the leaves were known, the amount of $CO_2$ produced per gram of dry weight could be determined.

Photosynthesis was calculated to be the difference in the amount of carbon dioxide in the effluent air from leaves in the light and in the dark. It was assumed that the respiration rate was the same in the light or dark.

Ozone and ozonated hexene were prepared as described previously (8), except that the gas mixtures were passed into a bell jar and a Dynapump then removed the air from the bell jar and pumped it into the leaf containers at known rates of flow. For the ozone treatment of bean leaves, the airflow over the leaves averaged 22.7 l/hr (range for all experiments 15.5 to 38.8 l/hr); for ozone on citrus leaves the flow was 69 l/hr (range 66 to 72 l/hr).

The oxidant concentrations for leaves treated with ozone were determined by passing the gas sample through 2 % buffered KI solution (0.1 M phosphate buffer, pH 7.0). The amount of iodine released by the ozone was measured with a Beckman Model DU spectrophotometer at 360 mμ. For the ozonated hexene gas the amount of oxidant was measured by the thiocyanate method of Todd (7). In the ozonated hexene fumigations, the airflow averaged 43.4 l/hr (range, 13 to 84 l/hr); the amount of ozone introduced averaged 5.9 ppm (range, 1.1 to 15.8 ppm); and the amount of hexene introduced averaged 304.1 ppm (range, 51 to 490 ppm). The ratio of hexene to ozone averaged 98.1 (range, 6 : 1 to 445 : 1). Ratings of leaf damage were made at the end of each experiment, the extent of the damage being expressed as the amount of leaf surface affected.

For the measurement of oxygen uptake and the respiratory quotient of treated and normal leaves, plants were fumigated in a large fumigation space (187 cu ft) and immediately removed to the laboratory at the end of the fumigation. Leaf disks 14 mm in diameter were made with a cork borer. Twelve disks were placed in each Warburg flask. For oxygen measurements the flasks that were used contained 0.5 ml of 10 % KOH in the center well, which was equipped with a wick, and 0.5 ml water in the sidearm. For the study of total gas exchange, water was substituted for the KOH in the center well. The bath was kept at a temperature of 25° C, and readings were made every 30 minutes.

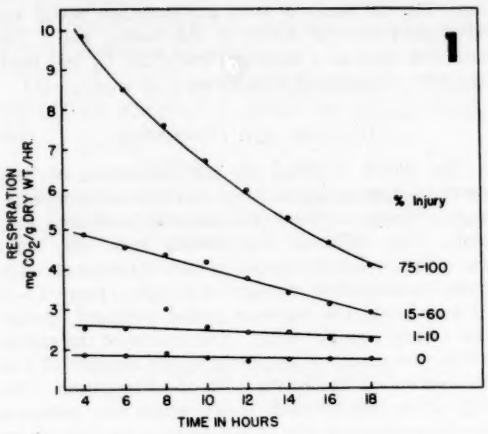
## Results and Discussion

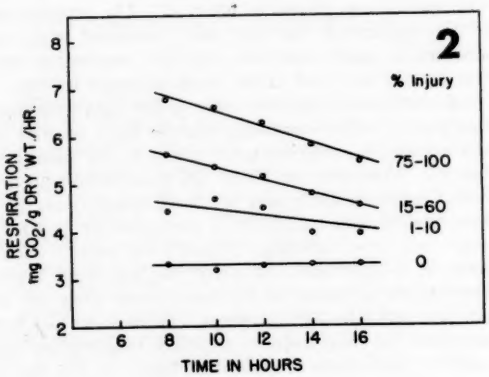
The effect of ozone on the respiration of bean leaves was investigated first, and various concentrations of ozone, exposure periods, and conditions were used. Nine different experiments were run, three sets of leaves being exposed in each experiment. The ozone concentration averaged 4.41 ppm (range 1.5 to 7.7 ppm), and the exposure period averaged 59 minutes (range 1 to 120 min). The results of the experiments were grouped according to the amount of visible damage on the leaves after the fumigation. The respiration rate of each injury group was corrected for the respiration rate of the controls for that group. The results are ploted in figure 1. The stimulation of the respiration rate was well correlated with the amount of injury observed, and the respiration rate was greatly increased in the badly damaged leaves.

A statistical study was made of the data presented in figure 1. The equations for the lines, as well as the regression coefficients, are given in the legend of figure 1. The line for 75 to 100 % damage contains both significant linear and quadratic components, and the experimental points fit a second-order parabola very well ($r = -0.9993$). Treated as straight lines, there is a significant difference (at the 0.001 level) between the slopes of all the lines except those for no damage and 1 to 10 % damage. Since these last two lines had the same slope, a statistical examination was made of the means of these two lines. It was found that they were not significantly different from a single line. Thus there was no significant response of the leaves unless the amount of injury exceeded 10 %, and the greater the leaf injury, the greater was the stimulation of respiration. No stimulation of respiration occurred when no injury developed. In the posttreatment period, the greater the stimulation, the faster was the decrease in respiration rate toward the pretreatment level.

A similar study was made of the effect of ozonated hexene on the respiration rate of bean leaves. Again a fairly wide range of concentrations and exposure periods was employed. Twenty-nine different experiments were run, and three sets of leaves were exposed in each experiment. The oxidant concentration (as measured with the thiocyanate reagent) averaged 1.87 ppm (range 0.5 to 7.2 ppm), and the length of the exposure period averaged 284 minutes (range 60 to 547 min). The results of the experiments were grouped according to the amount of injury observed. The respiration rate of each injury group was corrected for the respiration rate of the controls for that group. The results are plotted in figure 2.

The stimulation of the respiration rate was again correlated with the amount of injury observed (compare with fig 1 for ozone), and the maximum stimula-



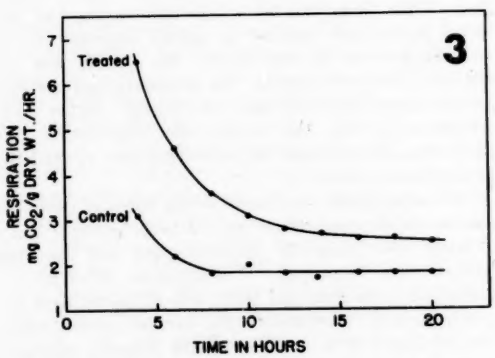




FIG. 1. Effect of ozone on the respiration of bean leaves. Equations calculated from the experimental points shown above: †

75 – 100 % injury—average of 4 runs
$Y = 10.95 - 1.26x + 0.05x^2$ $r = -0.9993$ ***

15 – 60 % injury—average of 3 runs
$Y = 5.20 - 0.29x$ $r = -0.9954$ ***

1 – 10 % injury—average of 3 runs
$Y = 2.79 - 0.07x$ $r = -0.6593$

tion of the respiration rate was about the same as for the ozone-treated leaves.

A statistical study was made of the data in figure 2. The data from all the injury levels appeared to fit straight lines very well. Comparison of the slopes of the various lines showed that the slopes of the 75 to 100 % injury and of the 15 to 60 % injury were not significantly different; nor were the slopes of the lines representing 15 to 60 % injury and 1 to 10 % injury significantly different. The slope of the line for 1 to 10 % injury was significantly different (at the 0.05 level) from that of the leaves not showing any injury. Assuming that there were no differences in the slopes of the various lines, it was found that the means of the lines were different. Thus, as when ozone was used in the fumigations, the greater the injury to the leaves, the greater was the stimulation of respiration. As with ozone, there was no respiratory stimulation in leaves that did not develop injury symptoms. However, regardless of the amount of the stimulation of respiration, the respiration returned to that of the pretreatment level at the same rate.

Bean leaves are very sensitive to both ozone and ozonated hexene, but some leaves, such as those of citrus, appear to be much more resistant to these gases. This raises the question as to whether the respiration rate of leaves that are not injured by ozone could be affected. Valencia orange leaves fumigated at a concentration of 2 ppm of ozone for 6 hours exhibited no change in respiration rate. From other fumigations it was known that citrus leaves could be

No injury—average of 3 runs
$Y = 1.89 - 0.02x$ $r = -0.7556$ *

*** Statistically significant at the 0.001 level.
* Statistically significant at the 0.05 level.
† x = time in the above figure; for equations substitute x = 1, 2, 3, 4, 5, etc. instead of 4, 6, 8, 10, 12, etc. as used above.

FIG. 2. Effect of ozonated hexene on the respiration of bean leaves. Equations calculated from the experimental points shown above.†

75 – 100 % injury—average of 6 runs
$Y = 7.21 - 0.34x$ $r = -0.9892$ **

15 – 60 % injury—average of 8 runs
$Y = 5.90 - 0.28x$ $r = -0.9972$ ***

1 – 10 % injury—average of 6 runs
$Y = 4.82 - 0.17x$ $r = -0.8115$

No injury—average of 7 runs
$Y = 3.25 - 0.01x$ $r = +0.9466$ *

*** Statistically significant at the 0.001 level.
** Statistically significant at the 0.01 level.
* Statistically significant at the 0.05 level.
† x = time in the above figure; for equations substitute x = 1, 2, 3, 4, 5, instead of 8, 10, 12, 14, 16 as used above.

FIG. 3. Effect of ozone on the respiration of Valencia orange leaves (average of 4 runs).

TABLE I

EFFECT OF OZONE AND OF OZONATED HEXENE ON THE UPTAKE OF OXYGEN BY DISKS FROM LEAVES OF FUMIGATED BEAN PLANTS (EACH VALUE THE AVERAGE OF DUPLICATES)

| FUMIGATION TREATMENT | | RESPIRATION AS % OF CONTROL * | | INJURY TO LEAF SURFACE (%) |
|---|---|---|---|---|
| MATERIAL | PERIOD (HR) | 2 HOURS AFTER TREATMENT | 26 HOURS AFTER TREATMENT | |
| Ozone ** | 0.5 | 119 | 138 | 15 |
| Ozonated hexene † | 0.5 | 129 | 125 | 57 |
| Ozonated hexene †† | 0.5 | 175 | 146 | 7 |
| | 1 | 184 | 174 | 17 |
| | 2 | 184 | 206 | 72 |
| | 4 | 161 | 208 | 83 |

* Actual respiration rate of control leaves ranged from 2.47 to 3.14 ml $O_2$/g dry weight/hr.

** Ozone concentration 0.44 ppm (KI).

† Ozonated hexene concentration 0.14 ppm (thiocyanate); 0.41 ppm $O_3$ introduced; 1.87 ppm hexene introduced.

†† Ozonated hexene concentration 0.15 ppm (thiocyanate); 0.55 ppm $O_3$ introduced; 5.20 ppm hexene introduced.

damaged by ozone at concentrations of approximately 15 ppm. The results of treating orange leaves with ozone at a concentration of 16 ppm are presented in figure 3.

None of the leaves that were treated showed any visible damage from the ozone, although the respiration rate in one experiment was stimulated to about four times that of the normal leaf. The average stimulation of the respiration rate after treatment was about twofold. The respiration of the treated leaves differed from the control respiration rate at the 0.01 statistical level. It should also be noted that following a long period in the light the respiration rate of both treated and control leaves was always higher than after a prolonged dark period.

The stimulation of citrus leaf respiration after exposure to ozone without the concomitant development of visible injury seemingly contradicts the data obtained from bean leaves, in which no stimulation of respiration occurred without the development of visible injury symptoms. This parallels the response obtained in experiments with lemon fruit (8), where a respiratory stimulation elicited by ozone was not accompanied by any obvious fruit injury. Since the concentration of ozone required for leaf response is close to that required for visible leaf injury, it is suggested that injury which is not visible to the naked eye does actually occur to some cells, but that the citrus leaf is able to repair the damaged cells.

Valencia orange leaves were also treated for 6 hours with ozonated hexene (up to 8 ppm oxidant as measured by thiocyanate), but no significant respiratory stimulation occurred. Greater concentrations of ozonated hexene were not tested, since 8 ppm was the maximum concentration obtainable with the fumigation equipment used.

All the results of respiration experiments presented thus far are based on the assumption that the carbon dioxide measured was actually respired by the leaves. To eliminate the possibility that the $CO_2$ could be produced by some process other than normal respiration, the uptake of oxygen by leaf disks taken from leaves of fumigated bean plants was measured. Table I shows that the increase in respiration rate as measured by oxygen uptake is similar to that measured with the $CO_2$ analyzer for both ozone and ozonated hexene. Bean plants were also treated for various lengths of time with ozonated hexene, and it can be seen from the data in table I that the stimulation of respiration was of the same order of magnitude for all of the series. This is in contrast to the results of other experiments in which respiration was measured by the infrared method and there was a correlation between amount of injury and stimulation of respiration. Perhaps more extensive experiments would give results similar to those of the other experiments.

Respiratory quotients for the bean leaf treatments described in table I were as follows: control, 0.98; ozone-treated, 1.02; ozonated hexene-treated, 0.97 ($\pm$ 5 %). The increase in both oxygen uptake and carbon dioxide evolved indicates that the usual substrates are being respired and that it is safe to assume that the carbon dioxide evolution as measured by the infrared method is related to the oxygen uptake in these experiments.

Since it was previously shown for Lemna (1) that photosynthesis could be greatly reduced in this plant by exposure to both ozone and ozonated hexene, it was of much interest to measure the response of photosynthesis in bean leaves to these gases. Photo-

TABLE II

INHIBITION OF PHOTOSYNTHESIS OF PINTO BEAN LEAVES BY OZONE- AND OZONATED HEXENE-TREATMENTS †

| INJURY TO LEAF SURFACE (%) | % INHIBITION OF PHOTOSYNTHESIS †† | |
|---|---|---|
| | OZONE | OZONATED HEXENE |
| 75–100 | 76.9 ** | 44.9 ** |
| 15–60 | 19.7 | 14.6 ** |
| 10 | 8.4 | 6.4 |
| None | −4.9 | −4.4 |
| Untreated | −6.0 | 1.7 |

† Actual rate of photosynthesis of leaves before treatment was (average) 38.8 mg $CO_2$/g dry weight/hr for ozone experiments and 26.2 mg $CO_2$/g dry weight/hr for ozonated hexene experiments.

†† Inhibition of photosynthesis was calculated by the following formula:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{Photosynthesis before treatment}}{\text{Photosynthesis after treatment}}\right) \times 100.$$

** Significantly different from the controls at the 0.01 statistical level.

synthesis was also determined in the experiments for which the respiration data is given in figures 1 and 2. It appeared that once the rate of photosynthesis was reduced, it remained at that level. The effects of ozone and of ozonated hexene on photosynthesis (table II) show inhibition as measured 14 to 20 hours after the start of the fumigation. The amount of inhibition of photosynthesis paralleled the extent of visible leaf damage. The photosynthesis of leaves that were injured to the extent of 75 to 100 % by either ozone or ozonated hexene was significantly reduced from that of control leaves. It should be noted that the extent of injury to the bean leaves by either ozone or ozonated hexene cannot be compared directly, since the ozone generally caused nearly complete destruction of the affected area, whereas the effect of ozonated hexene was seemingly limited to fewer layers of cells. This is borne out by a comparison of the reduction of photosynthesis by the two gases. For an injury level of 75 to 100 %, the photosynthesis of ozone-treated leaves was inhibited 77 %, whereas a similar injury level with ozonated hexene reduced photosynthesis only 45 %. Thus a measurement of photosynthetic activity of a leaf is undoubtedly a better index of the amount of permanent injury than is a visual estimate of the leaf injury. No change in the rate of photosynthesis was observed after the treatment of citrus leaves with 1 ppm of ozonated hexene.

It has been shown (3, 6, 9) that ozonated hexene can cause a decrease in plant growth which is not accompanied by visible injury to the leaves. It is doubtful whether this decrease could be attributed to an inhibition of photosynthesis since there was no effect on photosynthesis until injury occurred. There is the possibility that a stimulated respiration without any concomitant injury could have some long-range effect on growth. The greatest increase in respiration in citrus was 3.4 mg $CO_2$/g dry weight/hr, while the leaves photosynthesized at the rate of 13 mg $CO_2$/g dry weight/hr. This could conceivably upset the balance and use up carbohydrate needed for growth. A seemingly more likely explanation of the effect of ozonated hexene in decreasing growth without visible injury is that the growth process itself is affected directly (9), rather than indirectly by a reduction in the supply of available carbohydrate.

## Summary

The treatment of Pinto bean leaves with either ozone or ozonated hexene gas in air caused a rise in the respiration rate up to more than four times that of untreated control leaves. Plant responses to the two gases were similar. The rate of photosynthesis of treated bean leaves was less than that of controls. Both the amounts of stimulation of respiration and the inhibition of photosynthesis were directly related to the development of visible injury symptoms in certain tissues of the bean leaves. No stimulation of bean leaf respiration or inhibition of photosynthesis occurred unless visible injury developed. However, ozone caused a rise in the respiration rate of Valencia orange leaves with no concomitant development of visible injury. Citrus leaves gave no response until ozone concentrations approached those required to produce visible injury symptoms. Treatment with ozonated hexene at concentrations eliciting changes in respiration and photosynthesis in bean leaves caused no change in these processes in citrus leaves. A comparison of ozone- and ozonated hexene-treated bean leaves showed that the respiratory quotients were not different from that of the control leaves.

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# IODINE AS A MICRONUTRIENT FOR TOMATOES[1]

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Several studies made during the early years of this century (1, 2, 13, 15, 16) indicated that iodine has a stimulating effect on plant growth and may even be essential to it (10). Later work (3, 6, 8, 9, 11, 18) failed to confirm these findings conclusively, and as a result iodine is not yet recognized as an essential plant nutrient. Literature on the subject published up to the end of 1949 has been exhaustively reviewed by the Chilean Iodine Educational Bureau (5) who point out that contradictory or irreconcilable findings can to a large extent be explained on the grounds of differences in the form of iodine applied, species of plant, kind of soil, and concentration of the iodine solution or dressing used by the different experimenters who have examined this problem.

However, all are agreed, especially those who have been concerned with water culture experiments, that high concentrations are either harmful or have a depressing effect on plant growth. Only when the concentration is reduced to one part of iodine in 5 or 10 million parts of solution has any favorable stimulatory effect been observed.

Unfortunately, very few experiments have been carried out at these high dilutions and it is therefore desirable that further research should be devoted to concentrations of this order (5). Point is given to this conclusion by recent investigations showing that iodine is required in minute amounts for optimal growth of excised tomato roots in White's medium (7, 14, 17). Present evidence indicates that the micronutrient requirements of excised roots are qualitatively similar to those of whole plants.

The question of the value of iodine for plant life is not purely a theoretical one. Various market gardeners in England have found that tomato plants produce higher yields of fruit, ripening at an earlier stage, after the application of iodine compounds to soils and foliage. It is noteworthy that in these instances relatively large amounts of iodine are applied, a fact seemingly at variance with the conclusions reached from physiological investigations.

To add to our knowledge of the effect of iodine on plant life we undertook an investigation during the years 1952 to 1955 which comprised the following experiments on tomatoes.

A. Sand-water cultures: 1952, with and without KI; 1954, with and without KI and iodine; and 1955, with various iodine compounds in both the absence and presence of chlorine.

B. Soil cultures, principally with elemental iodine, either in Lugol's solution or dissolved in isopropyl alcohol: 1952, on a sandy soil poor in organic matter; 1953, on a sandy soil with a higher content of organic matter; and 1954, on a sandy soil and a loamy soil.

[1] Received June 2, 1958.

## EXPERIMENTAL

A. SAND-WATER CULTURES: The experiments were carried out in 6-liter pots (11) filled with 6.5 kg of glass sand and watered during the day with 2 liters of culture solution using the constant drop method. The nutrient solution used in 1952 and 1954 contained the following per liter:

| *Macro-nutrient* | *Concn. in millimoles* | *Micro-nutrient* | *Concn. in micromoles* |
|---|---|---|---|
| $NaNO_3$ | 2.35 | Fe citrate | 167.0 |
| $KNO_3$ | 1.54 | $H_3BO_3$ | 46.0 |
| $K_2SO_4$ | 1.28 | $MnCl_2 \cdot 4\ H_2O$ | 9.1 |
| $Ca(NO_3)_2$ | 1.02 | $ZnSO_4 \cdot 7\ H_2O$ | 0.77 |
| $MgSO_4 \cdot 7\ H_2O$ | 0.90 | $CuSO_4 \cdot 5\ H_2O$ | 0.32 |
| $NH_4H_2PO_4$ | 0.68 | $H_2MoO_4$ | 0.12 |

In 1955 the nutrient solution used by Broyer et al (4) was adopted because of its chlorine supplements, which enabled us to choose the chlorine treatments required in our Experiment 3. It should be noted that the relative ionic ratios and absolute concentrations are very different from those of the solution of the 1952 and 1954 experiments.

The plants were topped after five flower clusters had formed.

*Experiment 1—1952:* During this year an exploratory trial was made consisting of a treatment with 2 mg of KI per liter of nutrient solution and a control, both in 12-fold replication. The tomato variety used was Ailsa Craig.

The initial development of the plants was good, but growth was arrested temporarily about two months after the start of the experiment owing to the onset of calcium deficiency. After altering the composition of the culture solution, which originally contained only 56 mg of calcium nitrate per liter and watering the plants with a saturated solution of calcium sulphate they recovered and resumed growth. The plants did not, however, produce a full crop of fruit. In comparison with the control plants those treated with KI showed increased tangential growth, reduced radial growth, lower plant weights and improved fruit formation as reflected in an additional mean yield of fruits of 177 g per plant (76 % increase).

*Experiment 2—1954:* This year's experiment involved: (a), a control without iodine; (b), treatment with KI (2 mg per liter of culture solution); and (c), treatment with iodine (1.53 mg per liter of culture solution).

The solution for treatment (c) was prepared by adding a sufficient quantity of a 5 % solution of iodine in isopropyl alcohol (Shell I.P.S. 1), and in order to make the treatments comparable, equal quantities of isopropyl alcohol were also added to the solutions used in (a) and (b).

TABLE I

RESULTS OF SAND-WATER CULTURE EXPERIMENT, 1954

| TREATMENT | MEAN FRESH WEIGHT | | | MEAN INCREASE IN LENGTH | MEAN INCREASE IN DIAMETER |
|---|---|---|---|---|---|
| | PLANT | STEM | LEAVES | | |
| | *g* | *g* | *g* | *cm* | *mm* |
| Control | 760 ± 54 | 417 ± 39 | 343 ± 25 | 121 ± 4 | 4.4 ± 0.8 |
| KI | 710 ± 47 | 351 ± 44 | 358 ± 39 | 127 ± 4 | 5.2 ± 0.9 |
| Iodine | 557 ± 18 | 287 ± 35 | 270 ± 52 | 121 ± 4 | 4.9 ± 0.1 |

The experiment was carried out in 7-fold replication and the tomato variety used was Devon Surprise. The amount and composition of the nutrient solutions initially were the same as in 1952 except that iron was added as $FeCl_3$. During the vegetative stage the potassium content of the solution was increased by 50 % and the magnesium content by 25 %.

A striking difference in color was noted between the control and the iodine-treated plants, the former being darker green. Both KI and iodine tended to decrease the fresh weight of the plants, mainly by lowering the weight of the stems, but the difference was only significant in the plants treated with elemental iodine (table I). As in 1952, treatment with KI resulted in increased tangential growth (not significant) but radial growth now also appeared to have increased (see, however, footnote to table XII).

TABLE II

RESULTS OF SAND-WATER CULTURE EXPERIMENT, 1954

| TREATMENT | MEAN FRUIT YIELD | MEAN NUMBER OF FRUITS PER PLANT | MEAN WEIGHT PER FRUIT |
|---|---|---|---|
| | *g* | | *g* |
| Control | 3454 ± 18 | 64 ± 7 | 54 ± 1 |
| KI | 4108 ± 2 | 65 ± 3 | 63 ± 1 |
| Iodine | 3352 ± 19 | 55 ± 3 | 62 ± 1 |

Fruit yield was slightly reduced by free iodine but a marked and significant increase (19 % over the control) was induced by KI. This increase was due mainly to a higher weight per fruit (table II).

*Experiment 3—1955:* In the preceding experiments chlorine had been largely excluded from the culture solutions since it was thought that it might interfere with the iodine effect. Guided by the investigations of Broyer et al (4) we included chloride and bromide in our 1955 experiment in addition to iodide. The comparison of different forms of iodine was made at a chlorine level of 7.2 mg of KCl per liter of nutrient solution which we expected to give optimal results. The scheme for this experiment is shown in tables III and IV. The nutrient solution had the same composition as that described by Broyer et al. The variety of tomato plant used was Ailsa Craig and the number of replications 7.

From table III it can be seen that 1 ppm of KI increased tangential growth in both the absence and the presence of chlorine and that radial growth was decreased by KI and iodine irrespective of the concentration. Less regular results were obtained with KBr and KCl.

Unfortunately the higher chlorine level appeared to have a depressing effect on plant growth and has, therefore, to be considered an unfavorable standard for comparison. Nevertheless, potassium iodide in the lowest concentration produced positive results both with and without chlorine, whereas potassium

TABLE III

RESULTS OF SAND-WATER CULTURE EXPERIMENT, 1955
MEAN LENGTH AND DIAMETER * OF STEMS ON 27-7-1955

| TREATMENT | SERIES WITHOUT CHLORINE | | | | SERIES WITH 7.2 PPM KCl | | | |
|---|---|---|---|---|---|---|---|---|
| | LENGTH | Δ ** | DIAMETER | Δ ** | LENGTH | Δ ** | DIAMETER | Δ ** |
| | *cm* | *cm* | *mm* | *mm* | *cm* | *cm* | *mm* | *mm* |
| Control | 84 | .. | 15.7 | .. | 84 | .. | 16.3 | .. |
| KBr (2.9 ppm) | 85 | +1 | 15.8 | +0.1 | 83 | −1 | 15.8 | −0.5 |
| KBr (11.6 " ) | 88 | +4 | 15.6 | −0.1 | 85 | +1 | 15.7 | −0.6 |
| KI (1.0 " ) | 90 | +6 | 13.9 | −1.8 | 91 | +7 | 15.0 | −1.3 |
| KI (4.0 " ) | 81 | −3 | 12.5 | −3.2 | 85 | +1 | 12.7 | −3.6 |
| Iodine (0.76 ppm) | .. | .. | ... | .. | 88 | +4 | 14.4 | −1.9 |
| Iodine (3.1 " ) | .. | .. | ... | .. | 86 | +2 | 13.0 | −3.3 |
| KCl (1.8 " ) | 86 | +2 | 15.2 | −0.5 | .. | .. | ... | .. |
| KCl (7.2 " ) | 84 | .. | 16.3 | +0.6 | .. | .. | ... | .. |

* = Diameter of stem between 4th and 5th leaves.
** Δ = Deviation from control.

TABLE IV

RESULTS OF SAND-WATER CULTURE EXPERIMENT, 1955

| TREATMENT | NUMBER OF FRUITS | MEAN WEIGHT PER FRUIT | MEAN FRUIT YIELD PER PLANT | Δ * | GRADE OF FRUIT | |
|---|---|---|---|---|---|---|
| | | | | | MARKET | SMALL |
| | | *g* | *kg* | *kg* | | |
| Control (without Cl) | 50.8 | 62.5 | 3.33 | ... | 36 | 15 |
| KBr (2.9 ppm) | 60.3 | 54.7 | 3.30 | −0.03 | 39 | 21 |
| KBr (11.6 " ) | 52.8 | 58.0 | 3.07 | −0.26 | 39 | 14 |
| KI (1.0 " ) | 58.3 | 62.9 | 3.67 | +0.34 | 37 | 21 |
| KI (4.0 " ) | 43.2 | 57.5 | 2.48 | −0.85 | 28 | 15 |
| KCl (1.8 " ) | 58.7 | 59.2 | 3.50 | +0.17 | 42 | 17 |
| KCl (7.2 " ) | 54.0 | 48.4 | 2.63 | −0.70 | 34 | 20 |
| KCl + KBr (2.9 ppm) | 57.7 | 56.4 | 3.25 | +0.62 | 40 | 18 |
| KCl + KBr (11.6 " ) | 55.7 | 64.0 | 3.57 | +0.94 | 42 | 14 |
| KCl + KI (1.0 " ) | 60.3 | 55.8 | 3.37 | +0.74 | 41 | 21 |
| KCl + KI (4.0 " ) | 44.7 | 59.3 | 2.55 | −0.08 | 33 | 12 |
| KCl + Iodine (0.76 ppm) | 58.2 | 47.9 | 2.78 | +0.15 | 39 | 19 |
| KCl + Iodine (3.10 " ) | 44.3 | 52.3 | 2.32 | −0.31 | 29 | 15 |

* Δ = Deviation from control or from standard KCl level.

bromide increased yields only in the presence of chlorine. No signs of chlorine deficiency were observed in this experiment, but it should be noted that apart from using demineralized water and reagent-grade salts no special measures were taken to exclude traces of chlorine, bromine or iodine from either the nutrient solution or the air.

B. SOIL CULTURES: The following experiments were carried out: 1952, on a poor humic sandy soil from Wageningen; 1953, on a humic sandy soil from Varsseveld (eastern part of Holland); and 1954, on a loamy soil from Hampshire, England, in use for horticultural crops.

Analysis of the soils gave the following values:

| | *Wageningen* | *Varsseveld* | *Hampshire* |
|---|---|---|---|
| pH | 5.8 * | 5.6 | 7.4 |
| P, citric | 90 | 30 | 214 |
| % nitrogen | 0.105 | 0.16 | 0.30 |
| % organic matter | 3.1 | 4.4 | 4.4 |

* Adjusted to pH 5.8 by addition of $CaCO_3$.

During 1952 and 1954 the experiments were carried out in 6-liter pots and during 1953 in 15-liter pots. The latter were filled with 15 kg of soil and the former with 6.5 kg. The pots were watered daily and maintained at 60 % of their water-holding capacity (12).

The fertilizer treatment in the various experiments is shown in table V.

N and K were applied in 5, 4 and 3 equal doses in successive years.

*Experiment 4—1952:* The experiment consisted of 24 pots, half of which received a dressing of iodine in the form of diluted Lugol's solution. The latter was applied at a rate equivalent to a weekly dressing of 11.1 mg of KI and 5.56 mg of iodine per pot. The variety of tomato plant used was Ailsa Craig.

One month after planting, the iodine-treated plants were larger and more slender than the controls. The more rapid tangential growth of the experimental plants accelerated cluster formation and caused earlier flowering. As in the case of Experiment 1, after about two months a temporary growth stagnation occurred due to calcium deficiency. The plants treated with iodine were especially affected. After treatment with a saturated solution of calcium sulphate the disturbance was overcome and by the end

TABLE V

FERTILIZER TREATMENTS, 1952 TO 1954

| | 1952 | 1953 | 1954 |
|---|---|---|---|
| N | 5.0 g ($NaNO_3$) | 4.0 g ($Ca(NO_3)_2$) | 3.0 g ($\frac{1}{2}$ $NaNO_3$) ($\frac{1}{2}$ $Ca(NO_3)_2$) |
| $P_2O_5$ | see K | 4.25 g (superphosphate) | 4.25 g (superphosphate) |
| K | 5.22 g ($\frac{2}{3}$ $K_2SO_4$) ($\frac{1}{3}$ $K_2HPO_4$) | 9.32 g ($K_2SO_4$) | 7.0 g ($K_2SO_4$) |
| $MgSO_4 \cdot 7\,H_2O$ | 1.1 g | 2.2 g | 2 g |
| Borax | 50 mg | 100 mg | 50 mg |
| $MnSO_4 \cdot 4\,H_2O$ | 200 mg | 400 mg | 200 mg |
| $CuSO_4 \cdot 5\,H_2O$ | 25 mg | 55 mg | 25 mg |
| $FeCl_3$ | ..... | 100 mg | 50 mg |
| Ammonium molybdate | ..... | 20 mg | 10 mg |

TABLE VI

RESULTS OF SOIL CULTURE EXPERIMENT, 1952

| DATE | MEAN LENGTH OF PLANTS | | |
|---|---|---|---|
| | WITH IODINE | WITHOUT IODINE | DIFFERENCE |
| | *cm* | *cm* | *cm* |
| 18th June | 59.1 | 49.5 | 9.6 |
| 2nd July | 91.3 | 81.0 | 10.3 |
| 10th July | 104.4 | 97.0 | 7.4 |
| 31st July | 105.5 | 102.5 | 3.0 |
| 6th September | 117.4 | 108.4 | 9.0 |

of the experiment the iodine-treated plants again showed a substantial increase in length (table VI).

Contrary to the results obtained in the sand-water culture experiments the fresh weight (stem + leaves) of the iodine-treated plants did not differ from that of the controls (table VII). The increased tangential development, reduced radial growth and increased fruit yield (14.4 %), however, are in complete agreement with the results obtained in the sand-water culture experiments. It is interesting to note that iodine not only increased the fresh weight of the fruits but also induced a considerable increase in the percentage of dry matter.

*Experiment 5—1953:* Since it was suspected that iodine in soil cultures also had an effect on the microbial activity and nitrogen economy of the soil an experiment was designed in which plants were harvested at different intervals in order to determine their nitrogen content and to make observations on the time of cluster formation, dates of flowering and fruit setting and on the yield of tomatoes. The experiment comprised 28 pots, half of which were treated with diluted Lugol's solution (same strength and amount per pot as in the preceding experiment). In both the experimental and control groups pots were harvested as follows: 2nd July, 2 pots at the emergence of 1st cluster; 9th July, 2 pots at the emergence of 3rd cluster; 8th September, 4 pots one week after 3rd top-dressing; and 31st October, 6 pots on terminating the experiment.

No significant differences were observed in the time of cluster formation or of flowering. Determinations of the fruit yield were not made.

Nitrogen estimations (table VIII), however, gave some interesting results. In this connection it should be mentioned that at the end of August some of the iodine-treated plants showed symptoms of dying off in the lower and middle leaves, especially those facing the glass wall of the greenhouse. The leaves discolored quickly and became brownish-black, then they rolled up and finally turned necrotic.

In the dead leaves the nitrogen content was 3.8 $\pm$ 0.05 % on a dry matter basis, whereas in the green leaves of the control or of the iodine-treated plants it varied from 2.16 to 2.91 %. On the 8th of September the total amount of nitrogen in the leaves of plants treated with iodine was 48 % greater than in the control plants, despite a difference in weight of only 15 % in favor of the iodine-treated plants. On the same date soil samples were taken from 10 pots in each group and the following marked difference in nitrate content was found between the treated and non-treated pots: 162.0 $\pm$ 14.7 ppm nitrate nitrogen for the iodine group; and 86.2 $\pm$ 6.2 ppm nitrate nitrogen for the control group. The average ammonium nitrogen content amounted to 4.9 ppm and 4.5 ppm for the iodine and the control groups, respectively.

It is very likely that in some way iodine can enhance the rate of nitrification in the soil (see Discussion), and that the symptoms of leaf burn already mentioned were caused by nitrate accumulation.

It was observed when harvesting the plants at intervals that those treated with iodine had developed a more extensive root system than the control plants.

*Experiment 6—1954:* This experiment was carried out on a loamy soil from Hampshire, England, and

TABLE VIII

EXPERIMENT ON HUMIC SANDY SOIL—1953. QUANTITIES OF NITROGEN IN AERIAL PARTS OF PLANT

| DATE | WITHOUT IODINE | | WITH IODINE | |
|---|---|---|---|---|
| | LEAVES | STEMS | LEAVES | STEMS |
| | *mg nitrogen per plant* | | | |
| At commencement | 6.0 | 1.8 | 6.0 | 1.8 |
| 2nd July | 45.6 | 21.4 | 119.2 | 35.5 |
| 9th July | 204.8 | 78.7 | 196.7 | 88.1 |
| 8th September | 517 $\pm$ 41 | | 767 $\pm$ 36 | |
| 31st October | 540 $\pm$ 65 | | 623 $\pm$ 85 | |

TABLE VII

RESULTS OF SOIL CULTURE EXPERIMENT, 1952

| TREATMENT | MEAN LENGTH | FRESH WEIGHT | NUMBER OF FRUITS | WEIGHT PER FRUIT | FRUIT YIELD | DRY MATTER IN FRUIT |
|---|---|---|---|---|---|---|
| | *cm* | *g* | | *g* | *g* | % |
| With iodine | 117 $\pm$ 1 | 488 | 36 | 32.58 | 1173 $\pm$ 27 | 6.5 |
| Control | 108 $\pm$ 3 | 486 | 34 | 30.37 | 1025 $\pm$ 49 | 3.9 |
| Difference | 9 $\pm$ 3 | 1.8 | 2.3 | 2.21 | 148 $\pm$ 56 | |
| LSD 5 % level | 6.9 | | | | 116 | |
| LSD 1 % level | 9.4 | | | | 157 | |

TABLE IX

RESULTS OF EXPERIMENT ON LOAMY SOIL, 1954

| TREATMENT | MEAN FRESH WEIGHT | | | MEAN INCREASE IN LENGTH | MEAN INCREASE IN DIAMETER |
|---|---|---|---|---|---|
| | STEMS | LEAVES | TOTAL | | |
| | *g* | *g* | *g* | *cm* | *mm* |
| Control | 148 ± 2 | 222.4 ± 16 | 370 ± 22 | 61 ± 2 | 3.8 ± 0.7 |
| Iodine spraying | 145 ± 5 | 219.3 ± 21 | 364 ± 36 | 62 ± 1 | 3.8 ± 0.7 |
| Iodine dressing | 142 ± 7 | 192.0 ± 7 | 334 ± 16 | 69 ± 3 | 4.0 ± 0.7 |

the tomato variety used was Devon Surprise. The experiment involved: (a) a control; (b) weekly spraying of plants with a dilute solution of iodine (one part of iodine in 20,000 parts of dilute isopropyl alcohol); and (c) dressing with iodine (application of 25 mg of iodine per pot every 2 weeks).

The general development of all the plants was good and during growth they did not show great differences. At the conclusion of the experiment, however, the plants dressed with iodine showed a greater increase in length while the weight of leaves in comparison with the control plants tended to decrease (table IX). Both dressing and spraying with iodine resulted in an increased yield of fruit (mean increase 8.5 %). (See table X.)

## DISCUSSION

The physiological experiments in sand-water cultures have shown that iodine applied as potassium iodide has a well-defined effect on the development and yield of tomatoes. The results, as shown in table XI, have been reproducible during the three years of experimentation.

It can thus be seen that iodine in the form of iodide induced accelerated growth of the stem but at the cost of radial growth and fresh weight of the vegetative parts. There also was some indication, especially in the 1952 experiment, that increased growth produced earlier flowering.

The plants treated with iodide gave increased fruit yields over the controls amounting to 76, 14.4 and 10 % in the three experimental years. In one experiment in which both the control and the experimental plants received chloride the increased yield from the iodide-treated plants was 28.5 %. Closer examination of the results showed that the increased yields were due mainly to an increase in the number of fruits, but in some cases to an increased fruit weight as well.

These results warrant the conclusion that iodine performs a physiological function in the plant. This raises the question whether this element activates some biochemical synthesis or forms part of a plant hormone which is possibly involved in the development and stretching of the cells.

We shall discuss later the possibility of an indirect effect being involved where soil cultures are concerned, but in the case of the sand-water cultures described, such an effect is not likely to occur. The possibility that iodine exercises an indirect effect by influencing the uptake or action of other elements is also unlikely. The effects of iodine were fairly reproducible in different years and, as far as our investigations showed, the trend was not dependent on the composition of the nutrient solutions.

As has been emphasized by the Chilean Iodine Educational Bureau (5) the concentration of iodine is of essential importance and determines whether the effect is beneficial or harmful. On equating the concentrations of potassium iodide used in our sand-water cultures to the effective volume of solution (1.8 liters in 6 liters of sand) we find that the concentrations obtained and their corresponding effects agree very well with those already reported by Wynd (15). (See table XII.)

The foregoing results show that above 1 ppm potassium iodide shows toxic or depressing effects but that below this level the effects are clearly beneficial.

TABLE X

RESULTS OF EXPERIMENT ON LOAMY SOIL, 1954

| TREATMENT | MEAN NUMBER OF FRUITS | MEAN WEIGHT PER FRUIT | MEAN YIELD PER PLANT |
|---|---|---|---|
| | | *g* | *g* |
| Control | 26 ± 1 | 47 ± 1 | 1233 ± 22 |
| Iodine spraying | 28 ± 1 | 48 ± 1 | 1344 ± 26 |
| Iodine dressing | 27 ± 1 | 50 ± 1 | 1332 ± 23 |

TABLE XI

EFFECT OF IODINE AS POTASSIUM IODIDE

| YEAR | LENGTH OF PLANT | WEIGHT OF PLANT | DIAMETER OF STEM * | YIELD OF FRUIT |
|---|---|---|---|---|
| 1952 | Increased | Decreased | Reduced | Increased |
| 1954 | Increased | Decreased | Increased* | Increased |
| 1955 | Increased | Not determined | Reduced | Increased |

* In the 1st two years of experimentation the diameter of the stems was determined about 3 cm above the base. This does not seem to be satisfactory since in 1954 with an increased length and decreased weight of stems the diameter of the treated plants appeared to be greater than that of the controls. In 1955, therefore, we determined the diameter between the 4th and 5th leaves.

TABLE XII

SAND-WATER CULTURE EXPERIMENTS

| | YEAR | CONCENTRATION OF KI | EFFECT OF IODINE |
|---|---|---|---|
| Wynd | 1934 | 20.0 ppm | Depressed growth |
| Present paper | 1955 | 1.2 " * | Depressed growth |
| Wynd | 1934 | 1.0 " | Depressed growth |
| Present paper | 1952 | 0.6 " * | Accelerated growth and Increased fruit yield |
| Present paper | 1954 | 0.6 " * | |
| Present paper | 1955 | 0.3 " * | |

* Our 6-liter pots contain as a rule 6.5 kg of sand having a water-holding capacity of about 1.8 liters. We have thus multiplied our real concentration by 1.8/6.

According to the review already cited (5) beneficial effects on other crops have usually been found below 0.25 ppm.

It can be stated here that the glass-sand used in our experiments was not completely free from iodine. In 1955 we determined a content of 0.01 mg of iodine per kg but this was completely water-soluble and cannot be considered to play a role in a continuous flow method.

According to analyses made in our laboratory by Dr. G. W. F. H. Borst Pauwels, the iodine content of our nutrient solutions was of the order of 0.001 ppm which is low in comparison with the amounts supplied in the various treatments. There is, however, reason to consider the atmosphere as a possible source of iodine for plants. From Experiment 6 it appears that this element can be directly assimilated by the leaves and therefore it seems to be necessary to carry out future experiments under conditions where the air can be made iodine-free. Such experiments are now being conducted by Dr. Borst Pauwels.

As far as we were able to determine in sand-water cultures (Experiments 2 and 3) iodine in the elemental form does not exercise the same effect as iodide or does so only to a lesser degree. In general elemental iodine tended to have a beneficial effect. This matter, however, is complicated since, in addition to a physiological effect on the plant, one has to account for interactions of iodine with the soil.

Different soil interactions can be considered in principle. The first concerns a sterilizing action by which pathogenic organisms (bacteria or fungi) are killed and the growth of algae is retarded, thus enabling a more healthy development of roots and plants to take place. No observations in this sense have been made by us and the only fact observed was a better root development in our 1953 experiment.

A sterilizing effect could also play a role when spraying plants with iodine and in horticultural practice one could possibly prevent or limit the occurrence of fungus diseases by such treatment. In our pot trials this possibility can almost certainly be excluded.

A 3rd influence via the soil microflora concerns a possible effect on nitrogen-fixing or nitrogen-converting bacteria, and it certainly played a role in at least one of our trials. In Experiment 5 arrest of growth occurred in the plants dressed with Lugol's solution shortly after the 3rd top-dressing with nitrogen and potash. At the same time abundant signs of burning of the lower and middle leaves appeared in some of the plants. As shown by chemical analyses the iodine-treated plants contained a much higher amount of nitrogen, especially in the burnt leaves. The soil treated with Lugol's solution contained an amount of nitrate-nitrogen which was nearly double that in the control pots. Similar results (to be published elsewhere), showing an influence of iodine on the activity of nitrifying bacteria, were obtained in control pots with uncropped soil.

It will be clear that the aforementioned effects will interfere with the purely physiological effect of iodine compounds on plants and that the results of pot trials on soil will be more unreliable than those obtained in sand-culture or water-culture experiments. If the correct quantity of iodine is applied, the physiological and microbiological effects probably will supplement one another but the latter certainly will be dependent on the kind of soil.

The results of the iodine trials on soils in relation to the control results are summarized in table XIII.

The characteristics observed during vegetative growth did not show a pronounced and regular picture, although the tendencies were the same as in the sand-water cultures. The influence of iodine on fruit yield, however, was always positive.

Although we feel that potassium iodide alone or in combination with elemental iodine produces more regular and certain effects than elemental iodine, appreciable beneficial effects were obtained with pure iodine on a loamy soil by both dressing and spraying. In the latter treatment, an effect on the microflora is almost certainly excluded and this means that elemental iodine can be assimilated directly by the foliage and also is physiologically active.

TABLE XIII

SOIL EXPERIMENTS, COMPARISON OF IODINE TREATMENTS WITH CONTROLS

| EXPERIMENT | FORM OF IODINE | LENGTH OF PLANT | DIAMETER OF STEM | WEIGHT OF PLANT | YIELD OF FRUIT |
|---|---|---|---|---|---|
| 1952: 4 | Lugol's solution | Increased | Decreased | Equal | Increased |
| 1953: 5 | Lugol's solution | Not done | Not done | About equal | Not done |
| 1954: 6 | Iodine dressing | Increased | Decreased | Decreased | Increased |
| 1954: 6 | Iodine spraying | Equal | Equal | Equal | Increased |

TABLE XIV

COMPARISON OF THE EFFECTS OF IODINE CONCENTRATION

| YEAR | AUTHORS | FORM OF IODINE | CONCENTRATION IN SOIL | RESULT |
|---|---|---|---|---|
| 1942 | Hageman et al | KI | 1 : 190,000 | No effect on growth |
| 1952 | Present authors | KI + iodine | 1 : 214,000 | Marked increase in fruit yield |
| 1954 | Present authors | Iodine | 1 : 240,000 | Marked increase in fruit yield |
| 1937 | McHargue et al | Iodine | 1 : 400,000 | Marked benefit to fruit yield and quality |

In our soil cultures the average daily supply of iodine was about half of that present in the nutrient solution used in our sand-water cultures. However, in the soil cultures, an accumulation of iodine occurred which increased with time, whereas the concentration in the sand-water cultures remained the same.

Taking as a measure the initial concentration in our pot cultures we can make a comparison with results reported in the literature (table XIV).

Thus it would seem that the higher concentrations are especially harmful to young plants but are harmless or may even be beneficial in the later stages of growth.

In our earlier trials chlorine was largely excluded and it might be considered that the poor growth in our sand-water culture experiment in 1952 was due to an insufficient supply of this element. The effect of chlorine was investigated only in our experiment of 1955 when no positive evidence was obtained that this halogen is essential for tomatoes. It should be pointed out, however, that unlike Broyer et al (4) we took no special precautions to exclude all traces of chlorine from our culture solutions.

## CONCLUSION

1. In sand-water cultures with tomato plants potassium iodide increased tangential growth at the expense of radial growth, but decreased plant weight. Plants treated with iodide tended to show accelerated cluster formation and earlier flowering. They also produced higher yields.

2. In soil cultures iodine applied as iodide improved the fruit yield, mainly by increasing the number of fruits but also partly by increasing fruit weight. In one of the experiments the fruits appeared to have an appreciably higher percentage of dry matter than the controls.

3. Where a comparison was made, iodide gave better results than elemental iodine but the latter was also effective in increasing the fruit yield in pot culture experiments.

4. It was confirmed that it is the concentration of iodine which determines whether iodine will have a harmful or a beneficial effect.

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# DISTRIBUTION OF BORON IN CELLS OF DICOTYLEDONOUS PLANTS IN RELATION TO GROWTH[1,2]

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Relatively little is known about the locus of boron in the plant cell or its availability for movement from one site to another. It appears that little or no reutilization of boron occurs in dicotyledonous plants, and an external available supply is needed throughout the period of growth. As the borate ion complexes readily with various polyhydroxy compounds (13), localization of boron in certain cellular fractions might not necessarily imply an important physiological role at these sites; nevertheless, information on its distribution or localization within the plant cell could contribute to a better understanding of its function.

Boron determinations on various intracellular fractions separated by differential centrifugation indicated certain consistent trends regarding the distribution of boron within the cell, particularly in relation to growth, boron utilization and boron availability. These results have been briefly summarized (12) and will be reported fully here.

## MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L., var. Mammoth Russian) and mung bean (*Phaseolus aureus* Roxb.) were grown in nutrient solutions in a controlled-environment room under conditions previously described (10). The seeds were sown in quartz sand, watered with tap water, and one week later the seedlings were transferred to nutrient solutions contained in wide-mouth, low-boron soft glass quart fruit jars wrapped in black cloth. The sunflower plants were grown in solutions of the macronutrient composition previously described (10); the solutions for the mung beans consisted of a one-eighth concentration of Hoagland and Arnon's no. 1 solution (3). Iron, manganese, zinc and copper were added to both solutions at concentrations of 5.0, 0.5, 0.05 and 0.02 ppm respectively.

As plants absorb boron somewhat in proportion to the amount available, boron may accumulate at certain sites in amounts greater than required, and it may also be fixed at sites where it is not normally required. For these reasons boron was supplied in amounts that did not exceed normal growth needs. Preliminary studies were conducted to determine the minimal amounts of boron required to maintain normal growth (free of boron deficiency symptoms) for a period of 18 days. These studies indicated that a total of 50 $\mu$g boron per plant for sunflower and 5 $\mu$g boron per plant for mung bean (supplied as $H_3BO_3$) was just sufficient for this period of growth under the environmental conditions indicated. At the 18th to 19th day under this regime the sunflower plants started to show symptoms of boron deficiency.

Plants were harvested at several periods for various determinations. Twenty-five g fresh tissue samples were used; this included the terminal bud regions and the blades of the two youngest, fully expanded leaves of about 11 plants for sunflower and the terminal bud regions and the leaflets from the youngest, fully expanded leaf of about 45 plants for mung bean. The samples were prepared and separated into various intracellular fractions by the procedure used by Gordon (2). The tissue was ground in a chilled mortar with the aid of acid-washed sand in 50 ml phosphate buffer, 0.2 M, of pH 7.0, made 0.3 M with sucrose. The homogenate was strained through 200-mesh nylon cloth; the material that passed through is designated homogenate filtrate and that remaining in the cloth as homogenate residue (table I). The various cellular components were separated from the homogenate filtrate by differential centrifugation (table II).[4] Centrifugations up to 17,000 × G were performed in a refrigerated model Sb-1 International centrifuge; the 105,000 × G centrifugation was carried out in a Spinco Model L preparative centrifuge.

Aliquot samples of the supernatant fraction remaining after centrifugation at 105,000 × G for 30 minutes were dialyzed (in cellulose tubing, Visking Company) against $1 \times 10^3$ volumes of distilled water at 3° C for 24 hours to remove the dialyzable boron.

Boron analyses were performed by the colorimetric method of Dible et al (1); readings were taken on a Beckman DU spectrophotometer at 540 m$\mu$. Dry weights of the samples were obtained after drying at 100° C for 24 hours and corrections were made for sand and added sucrose.

## RESULTS AND DISCUSSION

DISTRIBUTION OF BORON IN INTRACELLULAR FRACTIONS: Two exploratory trials were conducted which involved boron determinations by spectrographic analysis of various cellular fractions separated by differential centrifugation. These results were con-

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[2] This work was performed under the auspices of the U. S. Atomic Energy Commission and aided in part by Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[3] The major part of this work was completed while Dr. McIlrath was a Resident Research Associate at Argonne National Laboratory.

[4] Identification of these particulate fractions with the corresponding centrifugations had been made previously by Dr. S. A. Gordon employing light and electron microscopy supplemented with Feulgen and Janus Green B reactions. The mitochondrial fractions were able to carry on oxidative phosphorylation with a P/O ratio of ca. 0.9.

TABLE I

BORON CONTENT OF HOMOGENATE FRACTIONS OF LEAVES AND APICAL BUD REGIONS OF MUNG BEAN AND SUNFLOWER PLANTS *

| FRACTION | MUNG BEAN | | | | SUNFLOWER | | | |
|---|---|---|---|---|---|---|---|---|
| | DRY WT OF FRACTION (% OF BOTH FRACTIONS) | BORON CONC OF FRACTION (μG/G) | TOTAL BORON OF FRACTION (μG) | TOTAL BORON IN FRACTION (% OF TOTAL IN BOTH FRACTIONS) | DRY WT OF FRACTION (% OF BOTH FRACTIONS) | BORON CONC OF FRACTION (μG/G) | TOTAL BORON OF FRACTION (μG) | TOTAL BORON IN FRACTION (% OF TOTAL IN BOTH FRACTIONS) |
| Homogenate residue | 61.3 | 36.1 | 87.5 | 49.4 | 63.3 | 26.1 | 67.5 | 50.1 |
| Homogenate filtrate | 38.7 | 48.1 | 89.7 | 50.6 | 36.7 | 38.0 | 67.3 | 49.9 |

* 25 g fresh plant sample.

sistent with those of a third and major experiment which will be presented. The boron analyses of all but the two exploratory experiments were carried out by the colorimetric (curcumin) method.

The tissues for this set of analyses were obtained from a harvest made 18 days after the plants were transferred to nutrient solutions containing a total of 50 μg B (for sunflowers) and 5 μg B (for mung beans) per plant. In both the mung bean and sunflower samples the homogenate residues constituted slightly more than 61 % of the total dry matter and contained about 50 % of the total boron (table I). The filtrates had about the same amount of total boron but a smaller dry weight, and therefore contained a higher boron concentration.

Because of overlapping of particle sizes, it is not possible to attain an absolute separation of the various cellular components by differential centrifugation. Nevertheless, boron analyses on the centrifuge fractions (characterized by the indicated cellular components) (table II) showed a variation in boron content. The concentrations and total amounts of boron in the fractions containing plastids and nuclei and in the non-particulate cytoplasm (dialyzed supernatant) were considerably higher than those in the mitochondria or "microsomes." The relatively high concentrations of boron in the chloroplasts is in agreement with the observations of Otting (6) but opposed to those of Smith (9).

Smith (9) separated chloroplasts from squash leaf tissue and found them to contain relatively small amounts of boron; he concluded that this element could have no function in this part of the cell, whereas Otting (6), on the other hand, found considerably higher amounts of boron in chloroplasts. The possible significance of the marked difference between the boron content of the sunflower and mung bean "microsomes" fraction will be referred to later.

The relatively low level of boron in the mitochondria, a fraction which constituted the highest percentage of dry weight of the particulates, is of particular interest in the light of the importance of these structures as sites of enzymatic activity.

These determinations indicate that there is a meas-

TABLE II

BORON CONTENT OF INTRACELLULAR FRACTIONS OF HOMOGENATE FILTRATE OF LEAVES AND APICAL BUD REGIONS OF MUNG BEAN AND SUNFLOWER PLANTS *

| INTRACELLULAR FRACTION | MUNG BEAN | | | | SUNFLOWER | | | |
|---|---|---|---|---|---|---|---|---|
| | DRY WT OF FRACTION (% OF ALL FRACTIONS) | BORON CONC OF FRACTION (μG/G) | TOTAL BORON OF FRACTION (μG) | TOTAL BORON IN FRACTION (% OF TOTAL IN ALL FRACTIONS) | DRY WT OF FRACTION (% OF ALL FRACTIONS) | BORON CONC OF FRACTION (μG/G) | TOTAL BORON OF FRACTION (μG) | TOTAL BORON IN FRACTION (% OF TOTAL IN ALL FRACTIONS) |
| Cell debris 100 × G; 10 min | 41.1 | 22.9 | 16.0 | 30.0 | 16.0 | 32.4 | 8.4 | 21.2 |
| Nuclei and plastids 1,000 × G; 10 min | 8.8 | 63.7 | 9.6 | 17.9 | 15.3 | 32.4 | 8.0 | 20.2 |
| Plastids and nuclei 4,500 × G; 10 min | 13.0 | 40.3 | 8.9 | 16.7 | 18.8 | 28.7 | 8.7 | 22.0 |
| Mitochondria and plastids 17,000 × G; 30 min | 13.9 | 16.6 | 3.9 | 7.4 | 21.5 | 10.6 | 3.7 | 9.3 |
| "Microsomes" 105,000 × G; 30 min | 8.0 | 24.9 | 4.0 | 7.5 | 11.7 | 0.6 | 0.1 | 0.4 |
| Dialyzed supernatant | 15.2 | 41.3 | 11.0 | 20.5 | 16.7 | 39.0 | 10.6 | 26.9 |

* 25 g fresh plant sample.

urable difference in the distribution of boron within the cell, and this differential distribution could be related to physiological boron-dependent activities. There are, however, several possible alternative interpretations that would prevent drawing unequivocal conclusions in this regard, namely 1) the localization of boron may reflect the distribution and differential concentration of polyhydroxy compounds that complex with boron, 2) since the fractionations were carried out in an aqueous medium, some dissociation of the boron complexes may have occurred, and 3) nonspecific adsorption of boron by certain particulates may have taken place during the grinding and isolation procedures and therefore might not reflect boron distribution in an intact cell.

Boron of the Supernatant Fraction: The previous experiment indicates that there is an observable difference in the boron content of the various intracellular fractions. At the present time, however, no definite conclusions can be drawn as to the significance of these findings. A seemingly more significant relationship, however, was suggested when it was noted that certain changes in the boron level of the supernatant fraction took place and appeared to be related to boron availability and boron deficiency. An experiment was then carried out to examine these changes in more detail.

Sunflower plants, of sufficient numbers to provide daily harvest requirements over an extended period, were grown in nutrient solutions containing a total of 50 $\mu$g B per plant. Samples were prepared in the usual manner except that only the supernatant fraction remaining after centrifugation at 105,000 × G for 30 minutes was used. Boron determinations were made on aliquots of the total supernatant and on aliquots of dialyzed supernatant, thus providing boron analyses on 1) total supernatant, 2) nondialyzable supernatant (the supernatant aliquot after dialysis) and 3) dialyzable supernatant (by difference between 1 and 2).

Plants were harvested daily from the early part of the experiment until pronounced boron deficiency symptoms had developed. At the early part of the experiment when the plants were making normal growth, the nondialyzable boron represented about 25% and the dialyzable boron about 75% of the total supernatant boron (fig 1). As boron was exhausted from the nutrient solution, the dialyzable boron fraction of the supernatant dropped and finally reached zero when the plants exhibited pronounced boron deficiency symptoms at the terminal buds. Smith (9) also noted that the boron in the "vacuolar sap" declined to a very low level when the plants were boron-deficient. The nondialyzable boron fraction of the supernatant, however, remained constant throughout this time. These findings illustrate that the small amount of boron in the plant which is available for required plant functions is the unbound portion in the supernatant. When this is depleted, the plant exhibits the usual deficiency symptoms.

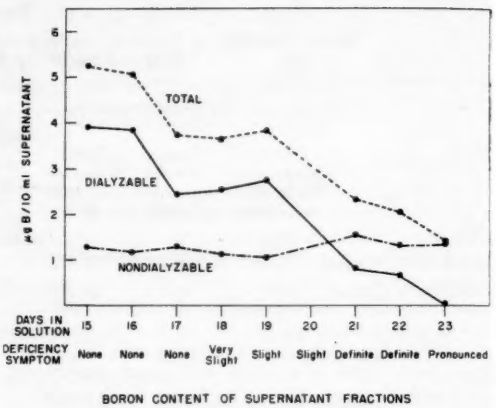


Fig. 1. Boron content of supernatant fractions (following centrifugation at 105,000 × G for 30 minutes) of sunflower tissue obtained from plants grown with a total of 50 $\mu$g B per plant for various periods.

The bound portion is apparently not available for movement to other sites. These observations also further substantiate various previous observations (e.g., 11) that boron is not reutilizable.

It appears that the dialyzable portion of the supernatant fraction represents free boron not yet utilized. Although it is of great importance to the economy of the plant, it may not serve any special physiological function in itself but perhaps represents an available pool for use in specific functions.

The physiological importance of the soluble boron fraction (cell sap obtained by mechanical pressure) in plants has been pointed out (4, 5, 7, 8) but this fraction has generally not been further separated. Shive (8) has shown that monocotyledonous plants contain greater amounts of soluble boron than do dicotyledonous species which is perhaps the reason why the former usually take longer to show pronounced deficiency symptoms.

Attention has been called to the marked difference between the boron content of the sunflower and mung bean "microsomes" fraction (table II). Sunflower plants grow considerably more vigorously than do mung bean plants and by the 18th day (time of harvest) and shortly thereafter, incipient boron deficiency symptoms generally appear in plants supplied with a total of 50 $\mu$g B per plant. Although the mung beans received one-tenth this supply, their rate of boron utilization is considerably slower and it is quite possible that they had not reached the same stage of incipient boron deficiency. The boron in the "microsomes" fraction may possibly be lightly bound and subject to release when the dialyzable supernatant boron level declines. If this is true it would be of considerable interest as other fractions of bound boron are apparently not available for further physiological utilization.

## Summary

Boron determinations were made on intracellular fractions separated by differential centrifugation. Mitochondria and "microsomes" were found to be lower in boron than nuclei, plastids and dialyzed supernatant fractions.

The boron content of the nondialyzable supernatant fraction does not change in response to decreasing amounts of available boron accompanied by the appearance of boron deficiency symptoms. The boron content of the dialyzable portion of the supernatant fraction, however, declines when supplies of available boron decline and it finally reaches zero in this fraction when plants exhibit pronounced boron deficiency symptoms.


We wish to thank Dr. S. A. Gordon for his valuable help and advice on the separations of the intracellular fractions by differential centrifugation and Messrs. J. K. Brody and J. A. Goleb for carrying out the spectrographic analyses.

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# ON THE ACTION OF PLANT GROWTH REGULATORS. II. ADSORPTION OF MCPA TO PLANT COMPONENTS[1]


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Plant growth regulators may be defined to suit the purposes of this paper as substances which at low concentrations give rise to increased cell elongation but at higher concentrations to phytotoxicity. The two effects may be quite distinct. The following paper offers a contribution to the knowledge of the mechanism of selective phytotoxicity in established plants.

Plants vary considerably in their resistance to growth regulators. Grasses and cereals generally, are resistant to low doses of 2-methyl-4-chlorophenoxyacetic acid (MCPA). Rape and mustard are highly susceptible whereas other species such as groundsel, chickweed and clover are intermediate. Resistant plants however respond to higher doses of MCPA. It may be assumed therefore, that a physiological effect can be produced in all plants provided the plant growth regulator reaches the appropriate site in sufficient quantity.

Resistance may result from one or more of the following causes: (a) low penetration of the solution into the plant; (b) adsorption losses during translocation from the leaf or stem to the site responsible for the physiological effect; (c) rapid destruction of the growth regulator by the plant; (d) an adverse effect on the permeability of cell membranes; and (e) low adsorption to sites responsible for the physiological effect.

In this work, adsorption processes are considered which could occur after the regulator has entered the plant. Thus it was shown (1) that there is a strong

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interaction between MCPA and proteins and to a less extent lipoproteins. It is thought that similar adsorption may occur in the plant to proteins and lipoproteins which play no part in the physiological response. Such adsorption might result in herbicidal resistance in some species, and other factors being equal, low adsorption to susceptibility in others. It is realized that this is not likely to be the sole cause of differences in species response.

The possibility that biological activity might be influenced by the adsorption of growth regulators to plant proteins has been considered by others in this field. Thus Wildman and Bonner (7) found that indole acetic acid (IAA) was firmly attached to protein fractions from spinach leaves. Moreover, there is evidence that two types of binding exist, giving rise on the one hand to stable complexes from which IAA cannot be removed by normal extraction procedure and the other, to complexes from which IAA may be removed by acetone or acid.

Veldstra and Booij (5) conclude that if the primary reaction takes place in the protoplasm, high adsorption to membranes might reduce the concentration of active material to levels at which it is ineffective. Leonard and Crafts (2) found that urea moved in the branches of live oak more rapidly than 2,4-dichlorophenoxyacetic acid (2,4-D), a fact they ascribed to the possibility of 2,4-D binding within the plant tissue.

Leopold (3) in a brief review alludes to work carried out in his laboratory in conjunction with Van Schaik. They measured the adsorption of various phenoxyacetic acids and benzoic acids to steam-killed plant leaves and claim that they obtained evidence supporting the contention that the resistance of corn to 2,4-D may be related to the ability of the tissue to adsorb strongly the herbicide on to places where it is not able to affect cell functions. It is not possible, however, to comment on this work as no experimental details or data are available.

No other attempt has been made to assess the extent of this binding or to relate it to the resistance of plant species. Veldstra and Havinga (6), however, from a study of force-area curves, concluded that the interaction between a number of plant growth regulators and a monomolecular layer of lecithin "came up to expectation." The author does not agree with this conclusion since it is not possible to obtain reliable estimations of adsorption without the use of surface potential measurements also.

The work described in this paper is a continuation of earlier work (1) in which it was shown that adsorption to components of wheat coleoptiles was approximately five times greater than that to those of cress and tomato stems. It aims at estimating the amount of adsorption of MCPA by components of a variety of plant species and to find if any correlation exists between these results and the resistance of plants to MCPA.

To measure the amount of adsorption, the work was subdivided: 1) the adsorption of MCPA to components of various plant species; and 2) determination of the amount of the adsorbing components in the plants.

## Materials and Methods

1. Adsorption of MCPA: *Apparatus:* A Langmuir trough and surface balance accurate to 0.2 dynes/cm were used to study the adsorption of MCPA to monolayers of components of the plant species. The apparatus consisted of a shallow glass trough filled with buffer solution, and equipped to measure the pressure and surface potential of a monolayer spread on the water surface as a function of the film area. The resulting curves, the force-area (F-A) and the surface potential-area ($\Delta V$-A), give information on the physical properties of the film and as seen later, enable the adsorption of MCPA to a film to be measured.

Surface potentials ($\Delta V$) were determined by means of a radioactive electrode in circuit with a triode valve electrometer. The latter was built from high grade resistances and potentiometers enabling steady surface potentials to be maintained for at least three hours. The trough was housed in a metal-lined box which was connected to ground and thermostatically maintained at 20° C.

*Experimental:* The preparation of the films from plant tissue was described in detail in the earlier paper (1). As it was impossible to ascertain the weight of material spread, standard conditions were maintained in different experiments by spreading material until a pressure of about 3 dynes/cm was built up. Moreover, as the molecular weight of the spread substances is also unknown, areas cannot be expressed in the usual units. Instead for simplicity, the distance "$l$" between the mica float of the surface balance and the moveable barrier, being proportional to the film area, was used.

To study the monolayer interactions of MCPA, (F-$l$) and ($\Delta V$-$l$) curves were plotted for monolayers on a pure substrate and compared with those from similar monolayers in equilibruim with a substrate containing MCPA. Adsorption of the injected acid was indicated by a change in surface potential (i.e., $\Delta V$ film $- \Delta V$ film/MCPA $= \delta\Delta V$) and/or an increase in surface pressure. Various concentrations of growth regulator were used in the experiments.

The growth regulator was injected in aqueous solution beneath the monolayer already on the surface. Gentle but prolonged stirring ensured that the substrate contained a uniform concentration. The substrates used were acetate buffers of pH 4.1, 4.3 and 4.7 and ionic strength I = 0.005.

All standard reagents used were of 'Analar' quality. MCPA was twice crystallized and had a melting point = 119° C. The water was distilled in an all-glass apparatus.

The following plant species were used: barley, *Hordeum sativum;* broad bean, *Vicia faba* (Prolific Longpod); chickweed, *Stellaria media;* cress, *Lepidium sativum* (Plain); groundsel, *Senecio vulgaris;*

maize, *Zea Mays* (Horsetooth); mayweed, *Anthemis cotula;* mustard, *Brassica alba* (white); oats, *Avena sativa* (Victory); onion, *Allium cepa* (Ailsa Craig); pea, *Pisum sativum* (Alaska); rape, *Brassica napus;* ryegrass, *Lolium italicum* (Italian); sugar beet, *Beta vulgaris* (Sutton's Improved); tomato, *Lycopersicum esculentum* (Sutton's Best of All); wheat, *Triticum vulgare* (Holdfast).

*Computation of results:* Three factors influenced the weight of acid adsorbed to a given system: (i) the amount of adsorbing material present (see appropriate section later in the paper); (ii) the number of adsorbing sites per unit area; and (iii) the degree of adsorption to the sites, referred to as "Extent or degree of adsorption, affinity".

The degree of adsorption to a monolayer was assessed in two ways. In the 1st method, $\delta\Delta V$ was assessed at a fixed concentration (C) of injected MCPA ($\delta\Delta V_C$). Provided $\delta\Delta V$ did not include contributions from accompanying film reorientations, it was directly proportional to the amount of acid adsorbed per unit area of film.

In the 2nd method, the percentage of adsorbing surface covered by a given concentration of injected MCPA was obtained from a Langmuir isotherm (1). No assumptions relating to film reorientations are involved in this method: it gives no absolute measure —merely the surface affinity for the injected acid.

In practice, both methods yield similar results. The 2nd was used in the earlier work of Brian and Rideal. It is now felt that for work involving only MCPA as injected acid, the 1st method is preferable in spite of the assumptions involved since it gives a more direct measure of possible adsorption within the plants.

2. AMOUNT OF ADSORBING COMPONENTS: Since total adsorption within a plant tissue is a function not only of the degree of adsorption but also the amount of adsorbing material present, the amount was estimated by the following procedure.

Plants were grown under similar conditions to those in Section 1 and were harvested for experimental purposes at the same stage of growth.

A weighed amount of plant tissue was ground in an ice-cooled pestle and mortar for five minutes with ice-cooled glass wool, which had been pretreated with chromic acid mixture to remove all traces of grease. The resulting slurry was then made up to volume (50 ml) with distilled water and sufficient redistilled absolute alcohol (both at 0° C) to give a final alcohol concentration of 20 %. The alcohol was added to facilitate the spreading of monolayers at a later stage in the technique. Finally, the solution was filtered through a no. 1 Whatman paper into a flask immersed in ice.

Further experiments were carried out in which the solution was filtered twice in order to ensure that removal of solid matter was complete. A 2nd filtration was found to be unimportant.

The filtrate was applied to the surface of a Langmuir trough with an Agla micrometer syringe. The volume required to form a film of fixed area (156 sq cm) at 2.2 dynes/cm was noted. Thus the total area of film produced by unit weight of tissue could be calculated as follows:

$$\text{Total film area} = \frac{156}{10{,}000} \times \frac{50}{v} \times \frac{1}{w} = \frac{0.78}{wv}\ \text{M}^2/\text{g}$$

where w = weight of tissue ground, v = volume of homogenate required to form a film of area 156 sq cm.

Dry weight determinations were also made on the plant material to enable the areas of film to be expressed on the basis of unit fresh weight and dry weight of tissue.

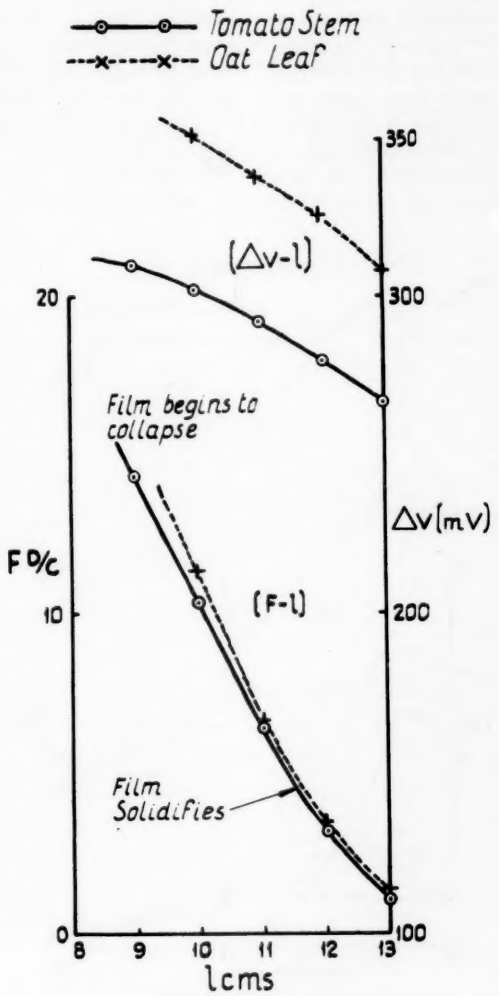



FIG. 1. The surface properties of tomato stem and oat leaf monolayers as measured by the change in pressure and surface potential with surface extension. pH 4.30 20° C

## Results

1. Adsorption of MCPA: Experiments were carried out at pH 4.1, 4.3 and 4.7. Since pKa for MCPA = 3.2, at pH values < 3.6, a proportion of undissociated acid would be present. To ensure therefore that the ionic form was mainly present, pH 4.1 was the lowest value used. At pH > 4.7, the interaction was small owing to ionic changes in the films themselves. pH 4.3 was found to be most satisfactory but most interactions were carried out at the three pH values.

Films were produced from the plant species listed in table II. They were all solid at pressures exceeding 4 dynes/cm. Like many protein films, they exhibited elastic properties in that talc scattered on the surface and displaced by a jet of air, returned to its original position when the air flow ceased.

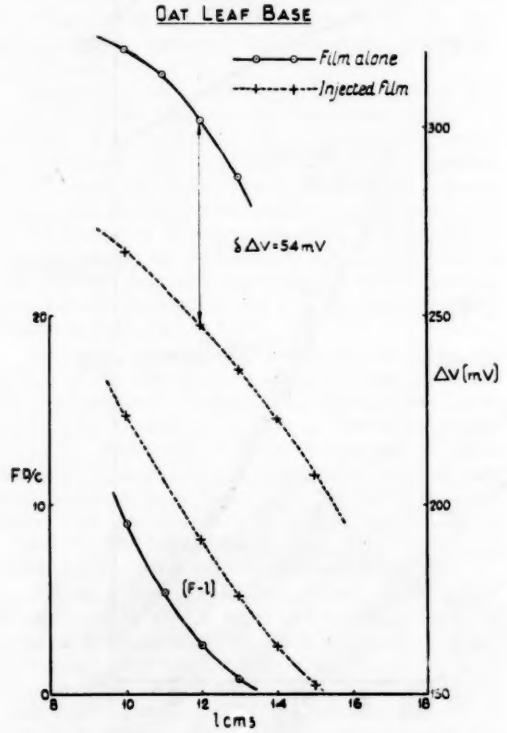
The surface potentials of the films at 3 dynes/cm were not widely variant, the lowest value being obtained from tomato stem (278 mV) and the highest from oat leaves (323 mV). The majority however lay between 300 and 320 mV. The (F-*l*) and ($\Delta V$-*l*) characteristics for tomato stem and oat leaf monolayers are shown in figure 1. Curves for monolayers of the other species listed under "material and methods" were similar in character.

MCPA was injected under the monolayers at a series of concentrations and at pH 4.1, 4.3 and 4.7. Figures 2, 3 and 4 present the (F-*l*) and ($\Delta V$-*l*) curves for oat leaves, tomato and groundsel stems at pH 4.3 before and after injection of $83 \times 10^{-5}$ M MCPA. ($166 \times 10^{-5}$ M for tomato stems).

It is seen that the injection of the organic acid not only expanded the monolayers but also reduced the value of $\Delta V$ in each case. The amount of reduction varied with the concentration of injected acid according to a Langmuir isotherm, figure 5. Isotherms for the monolayers from many of the tissues used are shown in figures 6, 7 and 8.

To compare the adsorption of MCPA to the monolayers from different plants, a point on a steep part of the isotherm was selected. A concentration $41 \times 10^{-5}$ M was taken to be the most suitable since at this point the curve is steep and the values derived appreciable.

The degree of reproducibility obtained by this technique is seen in table I where the change in surface potential produced by injecting $41 \times 10^{-5}$ M



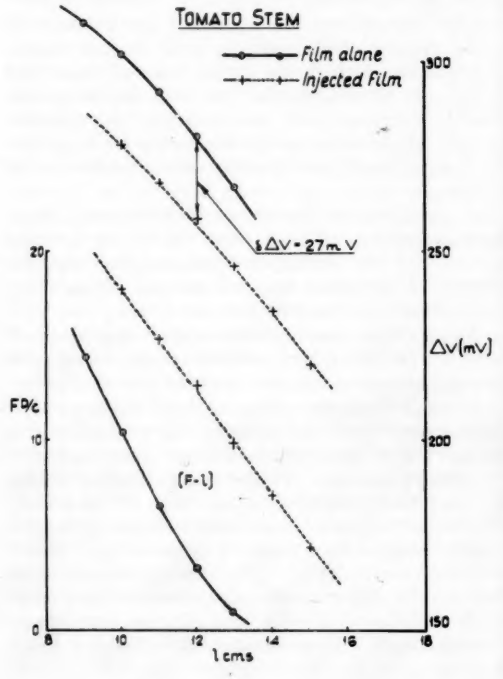




Fig. 2 (*left*). The change in pressure and surface potential with surface extension of oat leaf monolayers injected with $83 \times 10^{-5}$ M MCPA. pH 4.30 20° C

Fig. 3 (*right*). The change in pressure and surface potential with surface extension of tomato stem monolayers injected with $166 \times 10^{-5}$ M MCPA. pH 4.30 20° C

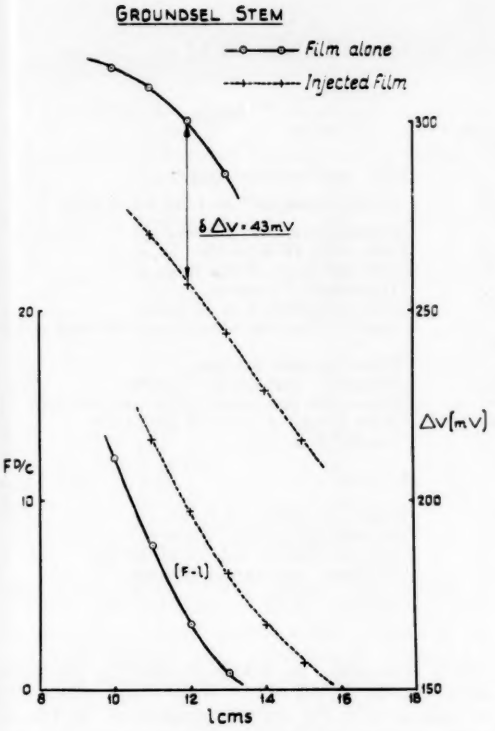



FIG. 4. The change in pressure and surface potential with surface extension of groundsel stem monolayers injected with $83 \times 10^{-5}$ M MCPA. pH 4.30 20° C

MCPA beneath oat monolayers at pH 4.30, is given for various experiments.

To show how the degree of adsorption of MCPA varies not only with plant species but also pH, table II gives the adsorption at pH 4.1, 4.3 and 4.7 to monolayers of the listed species, as measured by the fall in surface potential on injecting $41 \times 10^{-5}$ M MCPA.

It is seen that in all cases, the degree of adsorption falls rapidly with increasing pH though not to the same extent from species to species. At pH 4.3, the adsorption of MCPA by components of the above species varies significantly, high adsorption being given in all cases by the monocotyledons and the leaves of dicotyledons. Lower values were in general obtained for the stems or petioles of the dicotyledons.

TABLE I

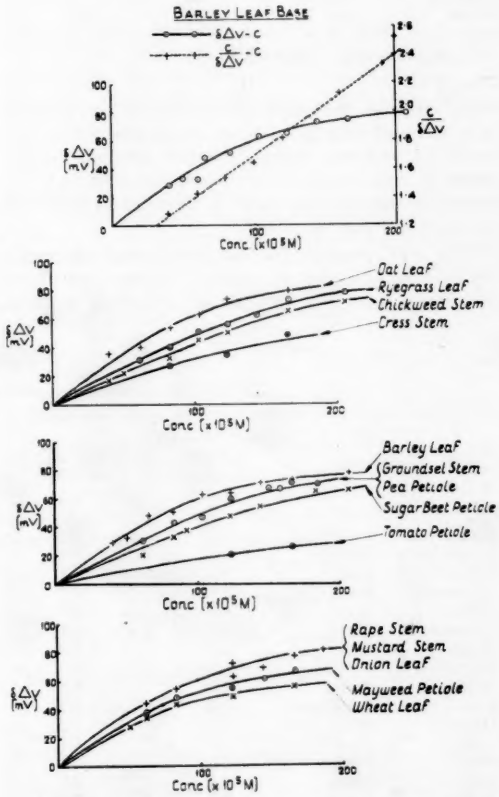
SURFACE POTENTIAL CHANGE BY $41 \times 10^{-5}$ M MCPA DESIGNATED $\delta\Delta V_{41}$ IN VARIOUS EXPERIMENTS

| DATE OF EXPERIMENT | | $\delta\Delta V_{41}$ (mV) |
|---|---|---|
| July | 1952 | 31 |
| October | 1952 | 31 |
| March | 1953 | 32 |
| May | 1953 | 29 |
| December | 1953 | 38 |
| November | 1954 | 35 |
| May | 1956 | 33 |

2. AMOUNT OF ADSORBING COMPONENTS: Experiments were carried out on separate batches produced from two or three plants of a given species (A, B, C, table III) and the results presented in table III are mean values of triplicate experiments on each batch. This table gives the amount of adsorbing components in various plant species expressed as square meters of film per gram of tissue.

The results indicate that with the exception of ryegrass and onion the leaves of monocotyledons and dicotyledons contained the highest quantities of adsorbing material. Considerable differences were found in the quantities obtained from the stems or petioles of the listed species but it is interesting to note that the portion of oats at seed level, like the leaves gave a high value.

It was not possible to make reliable estimations on groundsel and mayweed. In spite of working at 0° C, the amount of material adsorbed at the air/




FIGS. 5, 6, 7, 8. The adsorption of MCPA as evidenced by changes in surface potential resulting from the injection of varying concentrations of MCPA. pH 4.30 20° C

TABLE II

ADSORPTION OF MCPA TO VARIOUS PLANT SPECIES AT pH 4.1, 4.3 AND 4.7

| SPECIES | | ADSORPTION ($\delta\Delta V_{41}$) pH 4.10 | pH 4.30 | pH 4.70 | NOTES |
|---|---|---|---|---|---|
| Barley | Leaf base | .. | 29 | .. | Plant used at 4-leaf stage |
| Broad bean | Petiole | 29 | 15 | 8 | "Prolific Longpod" at 4- to 6-leaf stage |
| Broad bean * | Leaf | 39 | 30 | 17 | |
| Chickweed | Stem | .. | 23 | .. | Greenhouse grown, 4 weeks old |
| Cress | Stem | 23 | 14 | 11 | Plain cress, 10 days old |
| Groundsel | Stem | 36 | 24 | 16 | Field specimens, 8-leaf stage |
| Maize | Leaf base | .. | 26 | .. | "Horsetooth," 6 leaves |
| Mayweed | Petiole | 36 | 24 | 14 | Field specimens, 8 to 10 leaves |
| Mustard | Stem | Erratic results | 33 | 17 | Stable films but adsorption somewhat erratic |
| Oats | Leaf base | 42 | 28 | 21 | White mustard, 6 leaves<br>"Victory." Just before tillering |
| Oats | Seed level | 38 | 30 | 18 | Where root and young shoot join the seed |
| Onion | Leaf | 41 | 31 | 16 | "Ailsa Craig," 2 leaves, 6 weeks old |
| Pea | Petiole | .. | 22 | .. | "Alaska" 5 in. high |
| Rape | Stem | Erratic results | 30 | 12 | 6 leaves |
| Rape * | Leaf | .. | 34 | .. | |
| Ryegrass | Leaf base | 39 | 30 | 17 | Italian. 4 weeks old |
| Sugar beet | Petiole | 23 | 16 | 12 | "Sutton's Improved," 6 leaves |
| Tomato | Petiole | 20 | 6 | 2 | "Sutton's Best of All," 4 weeks old |
| Wheat | Leaf base | 32 | 25 | 18 | "Holdfast," just before tillering |

* Intervascular region only.

water interface decreased from replicate to replicate. It is assumed that breakdown of surface active materials by enzymes which occurred with all homogenates at room temperature, continued even at 0° C; hence the initial result only is recorded in table III for groundsel and mayweed.

Table IV presents the calculated total adsorption within a unit weight of tissues. Values quoted are the product of the amount of adsorbing material (table III) and the degree of MCPA adsorption $\delta\Delta V_{41}$ (table II, pH 4.3). While these values give no indication of the absolute amount of MCPA adsorption, since the number of adsorbing sites per unit weight of tissue is unknown, they do represent the relative amounts of MCPA which can be adsorbed to unit weight of the tissues studied.

Finally in table V total adsorption is reduced to the base tomato = 1, this species being selected be-

TABLE III

AMOUNT OF ADSORBING COMPONENTS IN VARIOUS PLANT SPECIES

| PLANT SPECIES | | FRESH WEIGHT GROUND TISSUE (G) | % DRY MATTER IN TISSUE | VOL HOMOGENATE USED (ML) A | B | C | MEAN VOL (ML) | AREA FILM/UNIT WT OF TISSUE ($M^2$/G) FRESH WT | DRY WT |
|---|---|---|---|---|---|---|---|---|---|
| Barley | Leaf base | 1.25 | 6.1 | 0.17 | 0.15 | ... | 0.16 | 3.9 | 64 |
| Broad bean | Petiole | 5.0 | 5.5 | 0.09 | 0.11 | ... | 0.10 | 1.6 | 28 |
| Broad bean | Leaf | 2.5 | 11.1 | 0.06 | 0.08 | ... | 0.07 | 4.5 | 40 |
| Chickweed | Stem | 2.5 | 4.3 | 0.18 | 0.16 | 0.17 | 0.17 | 1.8 | 43 |
| Cress | Stem | 4.0 | 5.2 | 0.30 | 0.27 | ... | 0.28 | 0.7 | 14 |
| Groundsel | Stem | 5.0 | 3.6 | 0.15 | 0.16 | 0.16 | 0.16 | 1.0 | 27 |
| Maize | Leaf base | 1.25 | 5.3 | 0.18 | 0.17 | ... | 0.18 | 3.5 | 66 |
| Mayweed | Petiole | 2.5 | 12.3 | 0.24 | 0.36 | 0.30 | 0.30 | 1.0 | 8.5 |
| Mustard | Stem | 5.0 | 6.7 | 0.25 | 0.19 | 0.24 | 0.23 | 0.7 | 10 |
| Oats | Leaf base | 1.25 | 8.1 | 0.09 | 0.08 | ... | 0.09 | 7.0 | 86 |
| Oats | Seed level | 1.25 | 7.5 | 0.08 | 0.12 | 0.10 | 0.10 | 6.3 | 84 |
| Onion | Leaf | 2.5 | 6.3 | 0.19 | 0.19 | ... | 0.19 | 1.6 | 26 |
| Pea | Petiole | 5.0 | 7.9 | 0.14 | 0.10 | 0.16 | 0.13 | 1.2 | 15 |
| Rape | Stem | 5.0 | 6.4 | 0.26 | 0.21 | 0.23 | 0.23 | 0.7 | 11 |
| Rape | Leaf | 2.5 | 7.9 | 0.035 | 0.035 | ... | 0.035 | 9.0 | 114 |
| Ryegrass | Leaf base | 2.5 | 11.5 | 0.15 | 0.14 | ... | 0.15 | 2.1 | 18 |
| Sugar beet | Petiole | 5.0 | 6.1 | 0.07 | 0.12 | 0.07 | 0.09 | 1.7 | 28 |
| Tomato | Petiole | 5.0 | 7.1 | 0.18 | 0.32 | ... | 0.25 | 0.6 | 8.7 |
| Wheat | Leaf base | 1.25 | 9.6 | 0.08 | 0.07 | ... | 0.08 | 7.8 | 81 |

TABLE IV

CALCULATED TOTAL ADSORPTION OF MCPA BY VARIOUS PLANT SPECIES.

| PLANT SPECIES | | DEGREE OF ADSORPTION OF MCPA | AREA OF FILM/UNIT WT OF TISSUE ($M^2/G$) | | TOTAL ADSORPTION | |
|---|---|---|---|---|---|---|
| | | | FRESH WT | DRY WT | PER UNIT FRESH WT | PER UNIT DRY WT |
| Barley | Leaf base | 29 | 3.9 | 64 | 113 | 1860 |
| Broad bean | Petiole | 15 | 1.6 | 28 | 24 | 420 |
| Broad bean | Leaf | 30 | 4.5 | 40 | 135 | 1200 |
| Chickweed | Stem | 23 | 1.8 | 43 | 41 | 990 |
| Cress | Stem | 14 | 0.7 | 14 | 10 | 196 |
| Groundsel | Stem | 24 | 1.0 | 27 | 24 | 648 |
| Maize | Leaf base | 26 | 3.5 | 66 | 91 | 1720 |
| Mayweed | Petiole | 24 | 1.0 | 8.5 | 24 | 200 |
| Mustard | Stem | 33 | 0.7 | 10 | 23 | 330 |
| Oats | Leaf base | 28 | 7.0 | 86 | 196 | 2410 |
| Oats | Seed level | 30 | 6.3 | 84 | 190 | 2520 |
| Onion | Leaf | 31 | 1.6 | 26 | 50 | 806 |
| Pea | Petiole | 22 | 1.2 | 15 | 26 | 330 |
| Rape | Stem | 30 | 0.7 | 11 | 21 | 330 |
| Rape | Leaf | 34 | 9.0 | 114 | 306 | 3880 |
| Ryegrass | Leaf base | 30 | 2.1 | 18 | 63 | 540 |
| Sugar beet | Petiole | 16 | 1.7 | 28 | 27 | 450 |
| Tomato | Petiole | 6 | 0.6 | 8.7 | 3.6 | 52 |
| Wheat | Leaf base | 25 | 7.8 | 81 | 195 | 2030 |

cause it had the lowest adsorption. These values are compared with the effect of MCPA on the established plants in the field in order to find if there is any correlation between the adsorption values found and resistance to MCPA of the plant species.

## DISCUSSION

The nature of the films spread directly from plant squashes is unknown. That they were reproducible was shown by repeat experiments using different plants as shown by an example quoted in table I using oats. They exhibited properties consistent with those of proteins or lipoproteins. Thus their surface potential fell with increasing pH and they possessed elastic properties.

It is assumed that the adsorption of MCPA to the monolayers was reversible and occurred through electrostatic forces reinforced by Van der Waals interactions. The specific inhibition of carboxypeptidase by certain carboxylic acids was found by Smith, Lumry and Polglase to be a similar adsorption process (4).

The films from all plant species were similar but not identical in properties. Thus the adsorption of MCPA decreased with increasing pH but not quite to the same extent from species to species. This reduced adsorption is consistent with the model proposed in the earlier paper by Brian and Rideal (1). There the fall was associated with the progressive ionization of –COOH groups in proteins and lipoproteins with increasing pH, giving rise to repulsion between the surface and the growth regulator anion.

In considering these results, it should be appreciated that the values given for total adsorption are relative, rather than absolute. It is possible the grinding of the tissue failed to bring all the adsorbing material into colloidal suspension, and that there were further losses on filtration. The technique for determining the amount of adsorbing material was, however, standardized throughout and it is reason-

TABLE V

COMPARISON BETWEEN TOTAL ADSORPTION OF MCPA AND ITS TOXICITY ON ESTABLISHED PLANTS

| PLANT SPECIES | | MCPA ON ESTABLISHED PLANTS IN THE FIELD | TOTAL ADSORPTION BASED ON TOMATO = 1 | |
|---|---|---|---|---|
| | | | FRESH WT | DRY WT |
| *Stems or Petioles* | | | | |
| Tomato | Petiole | Susceptible | 1.0 | 1.0 |
| Cress | Stem | " | 2.8 | 3.8 |
| Rape | Stem | " | 5.8 | 6.4 |
| Mustard | Stem | " | 6.4 | 6.4 |
| Broad bean | Petiole | " | 6.6 | 8.0 |
| Groundsel | Stem | Moderately resistant | 6.6 | 13.0 |
| Mayweed | Petiole | " | 6.6 | 4.0 |
| Pea | Petiole | Susceptible | 7.2 | 6.4 |
| Sugar beet | Petiole | " | 8.0 | 9.0 |
| Chickweed | Stem | Moderately resistant | 11 | 19 |
| Oats | Seed level | Resistant | 53 | 50 |
| *Leaves* | | | | |
| Onion | Leaf | Susceptible | 14 | 16 |
| Ryegrass | Leaf base | Resistant | 18 | 10 |
| Maize | Leaf base | " | 25 | 33 |
| Barley | Leaf base | " | 31 | 36 |
| Oats | Leaf base | " | 54 | 46 |
| Wheat | Leaf base | " | 54 | 40 |
| Broad bean | Leaf | ..... | 38 | 23 |
| Rape | Leaf | ..... | 85 | 74 |

able to assume that results for all species would be similarly affected. For these reasons, differences in total adsorption between species quoted in table IV and V are not treated quantitatively.

Film areas and hence total adsorption are calculated on the basis of unit fresh weight and dry weight of plant tissue. In spite of the difficulties of standardization inherent in fresh weight determinations, these results are preferred, because since they involve the amount of adsorbing material in the dry weight fraction, and also the amount of dry material in the tissue, they give a more realistic value for the total adsortpion. In practice, results calculated on either basis are similar and only three species appreciably changed in their relative adsorption. Thus on a dry weight basis, groundsel stem was more adsorbing while mayweed petiole and ryegrass leaf base became relatively less adsorbing. Minor changes such as the increase in oats adsorption relative to wheat are of no consequence in the discussion following. As the results expressed on either basis are similar, it is proposed to discuss only those derived from fresh weights.

While it is unlikely that differences in species response result solely from any one factor, it is here considered in terms of adsorption losses during transport from the areas of application to the sites of action within the plant. The exact location of these sites is unknown and they may be present within all living cells or confined to specific tissues. Whichever system is involved, it can be envisaged that significant losses of MCPA may occur by adsorption to specific areas of cell membranes and to the soluble adsorbing components of the cytoplasm.

The squashes used in this work probably contain materials from all the living tissues present in the plant part used for testing. Therefore the adsorption values obtained can be considered to give some indication of losses due to adsorption within individual cells as well as those involved during the general transport through the plant.

It is also appreciated that the squashes were obtained from different plant organs, i.e., from leaves, petioles and stems, and the difference in total adsorption obtained could be merely a function of the different ages and structures of these organs. That this is only partly the case, is seen in table IV where variations in total adsorption are shown to be a function not only of quantity of adsorbing material present but also the adsorbing properties of the films themselves which vary a maximum of 6-fold between tomato petioles and rape leaves.

Nevertheless, the results in tables IV and V are discussed under two headings: 1, petioles and stems and 2, leaves.

1. Petioles and Stems: A broad correlation was found between adsorption in the petioles and stems and resistance of the plants to MCPA. Thus least adsorption was found in tomato petioles and cress stems, an interesting result in view of the high susceptibility of these species to MCPA.

Furthermore, oats, one of the most resistant plants studied, gave the highest adsorption, a region at seed level being studied where conditions approach more closely to those of the petioles and stems of dicotyledons.

Between these extremes, a number of susceptible species, rape, mustard, peas, sugar beet and broad beans gave rise to low adsorption. Chickweed, with moderate resistance to MCPA gave a higher adsorption value. It may be noted however that groundsel and mayweed with values similar to those of several susceptible species, do not fall in line. These anomalies may be explained by the inability to obtain reliable estimates of the amount of adsorbing material present in these two species. Thus it was noted at table III that their monolayers were very unstable and hence estimates of the amount present in the plant material would of necessity be too low.

2. Leaves: Oats, wheat, rape and broad bean leaves contained large amounts of material which was relatively strongly adsorbing. Their adsorption of MCPA was greater than that of barley and considerably greater than that of ryegrass, maize and onion leaves. It is seen in table V that on a fresh weight basis, the leaves of all species studied adsorbed more MCPA than the petioles or stems of the dicotyledons.

It is interesting to observe that wheat and oats which are among the most resistant species, gave the highest adsorption of growth regulator (except rape). The lower adsorption by ryegrass is in line with its partial susceptibility particularly in the early stages of growth.

Onions being susceptible to MCPA, we believe that adsorption by their leaves, although less than that of the other leaves examined, is nevertheless greater than would be expected, and hence constitutes an exception to this correlation.

Thus high leaf adsorption (in dicotyledons, an intervascular region omitting all major vascular elements) was characteristic of not only the grasses, but also the dicotyledons. It implies that movement in the leaves may be restricted and two conclusions may be drawn. (a) Because of high adsorption in the leaves a small proportion only of the MCPA deposited on plants by sprays may be available to the plant from the leaves. (b) Resistance in grasses may result partly from the fact that spray deposits can fall only on the highly adsorbing leaves. In dicotyledons, however, MCPA falls also on the less adsorbing petioles and stem and may become available to the plant mainly in these regions. Preliminary work at Jealott's Hill (unpublished) indicates that species response is often markedly greater when MCPA is applied through the stem than through the leaves.

It is also suggested that having penetrated into the grasses further losses of MCPA occur during translocation, by immobilization at physiologically inactive sites. This is indicated by the results on petioles and stems where it is seen that plants giving monolayers which adsorb more MCPA, are more resistant in the field. Thus the work indicates that

resistance may be partly related to the presence of material within the plant which readily adsorbs the herbicide, so impeding its translocation through cells and conducting tissue.

Finally, it is of interest to consider an earlier observation (1) concerning lecithin. It is emphasized that this is purely speculative. Thus lipoid was found to reduce the adsorption of MCPA to protein monolayers, an effect that was ascribed to the neutralization of cationic groups on the protein by the negative phosphate ion of lecithin (presumably the positive ion of the lecithin is immobilized either by anions on the protein or by steric factors). As lecithin or related compounds are believed to occur in all plants, the amount present in each species may regulate the degree of MCPA immobilization in the plants. Thus it is reasonable to assume that as cationic groups adsorb MCPA anions, plants containing a higher proportion of lecithin etc. may adsorb less anions and hence be more susceptible to MCPA, and conversely, those containing less lecithin, or similar compounds, more resistant.

## Summary

Interactions between a plant growth regulator, 2-methyl-4-chlorophenoxy acetic acid (MCPA), and monolayers produced directly from plant tissues were studied at pH 4.1, 4.3 and 4.7.

Estimates were made of the amount of adsorbing material present in the various tissues and thus "total adsorption" of MCPA to selected organs from 16 plant species, was calculated.

A broad correlation was found between the resistance of a plant to MCPA and its ability to adsorb the growth regulator thus lending support to the suggestion that the foundation of species resistance may be partly based upon the extent of MCPA adsorption to sites not concerned in the physiological response.

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# FREE AMINO ACID LEVELS IN NICOTIANA GLAUCA, N. LANGSDORFFII AND THEIR INTERSPECIFIC HYBRID FOLLOWING GAMMA IRRADIATION[1,2]

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Morphological responses of *Nicotiana glauca*, *N. langsdorffii* and their hybrid *N. glauca* × *N. langsdorffii* to chronic gamma irradiation, include leaf abnormalities (3, 7, 12) similar to the disease of tobacco known as frenching. In an attempt to determine, in part, if like physiological changes occur in the two species and the species hybrid, free amino acid determinations were made of the above three at varying dosages of gamma irradiation. Since the two parents and the hybrid show differing degrees of sensitivity to radiation with respect to leaf changes, *N. glauca* being the most and *N. langsdorffii* the least sensitive with the hybrid intermediate, then morphological and physiological comparisons of sensitivity to radiation can be made. The hybrid, additionally, shows tumor formation as a radiation response (11). Comparisons, therefore, of free amino acids of tumor vs normal tissue are also made.

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[2] Research carried out in part at Rutgers, the State University, under A.E.C. contract no. AT(30-1)-1120 and with the cooperation of the University Research Council, and in part at the Brookhaven National Laboratory under the auspices of the A.E.C.

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## Materials and Methods

Planting: The tobacco plants used here were started in pots in the greenhouse at staggered intervals so that all plants would approximate the same physiological age when collections were made. Planting dates were as follows: *Nicotiana glauca* — March 15,

1956; *N. glauca* × *N. langsdorffii*—May 15, 1956; *N. langsdorffii*—May 29, 1956. However, it was found that even with these altered starting dates the langsdorffii parent flowered soon after being put into the gamma-field, and the hybrid began to flower just prior to collections. The glauca parent remained vegetative at all levels of radiation during the course of this experiment. Plants were placed in the gamma-radiation field at the Brookhaven National Laboratory in early July. Irradiations were made according to the methods described by Sparrow and Singleton (13). The plants received 400, 325, 250, 200, 150, 100, 50, and 25 r per 20-hour day, or no irradiation, with 10 to 15 plants being used for each treatment. After two months in the gamma-field, morphological changes were evident in all three plant groups receiving irradiation (3, 7, 12). Samplings were made at this time.

SAMPLING: Collections were made of leaves from all plants at all dosages, and tumors from hybrid plants receiving 300 r per day. Tissues were immediately weighed and extracted with ethanol to give 80% solution in the extract (2 cc/g of tissue) in a Waring blendor. The homogenate was filtered and the debris washed four times with 80% ethanol (1 cc/g/wash). The alcohol fractions were collected combined and stored in a refrigerator until used for chromatography.

CHROMATOGRAPHY: Free amino acids were concentrated from the stored samples in the aqueous phase of the extract-chloroform mixture (1:3) (1, 5). Depending upon the volume of the aqueous phase above, the water layer was made up to a volume of 1.5, 2 or 3 cc per g fresh weight of tissue with distilled water. For each of the adjusted volumes of tissue extract appropriate volumes of this extract were spotted on Whatman no. 3 filter paper. Chromatograms were run two-dimensionally; phenol : water (4:1) the 1st direction and butanol : acetic acid : water (9:1:1.8) the 2nd direction and sprayed with ninhydrin (10). Spots were cut out, weighed, cut into small pieces, and eluted with 50% ethanol in phosphate buffer (pH 7) for 10 hours. Color density of the eluent was measured with a Coleman Junior spectrophotometer at 570 mμ. Standards were determined according to Thompson et al (19) and Porter (10). Deviations from the standard curve varied from 10 to 20%, depending upon the amino acid in question.

## RESULTS

N. LANGSDORFFII: Figure 1 (upper left) represents the concentrations of free amino acids and amides at 8 dosage levels and in control tissues. Seven amino acids and amides were found at every dosage and in control plants, while asparagine made a trace appearance in plants irradiated at 200 r per day with measurable amounts present at 400 r per day. It is evident that not only do the concentrations per gram fresh weight of the acids increase above 150 r per day, but also the concentration of one acid with respect to another changes.

N. GLAUCA × N. LANGSDORFFII: Figure 2 (lower left) shows that in controls the same amino acids are found as in langsdorffii, and similar concentrations of these acids also exist. Here, however, the initial increase occurs above 50 r per day, that is, at a considerably lower dosage than in the langsdorffii parent.

N. GLAUCA: In the glauca parent, as seen in figure 3 (upper right) the free amino acid concentration in control tissues is considerably higher in total concentration than in the other two plants studied. Also, additional amino acids are found, and the initial increase in concentration of these acids, except for proline, occurs at 25 r per day.

HYBRID TUMOR TISSUE: Tumor tissue from plants at 300 r per day was collected, with two of the morphological tumor types (4), green-leafy and stalked, used for determinations. In addition, old tumor tissue from plants growing in the greenhouse was examined for free amino acids. It seemed desirable to compare old tissue, which was not growing rapidly, with rapidly growing tissue from the gamma-field to see if any significant differences in free amino acid content were detectable. Both tumor groups, old and young, had been induced by gamma radiation. Figure 4 (lower right) is a comparative graph of amino acid concentrations in *N. glauca*, *N. langsdorffii* and the hybrid. The values plotted are for control leaf tissue, leaf tissue at a dosage level where glutamine and asparagine were high, the two young tumor types and old tumor tissue. When the amino acid levels of the young tumor types were compared to those of irradiated hybrid tissue, it was seen that the glycine content was reduced by one half, glutamic acid concentrations doubled and glutamine increased four-fold. The striking difference, however was the appearance of asparagine in high concentrations. Young tumor tissue had higher concentrations of asparagine than any of the leaf tissues examined. Old tumor tissue showed a drop in both asparagine and glutamine, while the other acids remained at almost the same level as that found in the rapidly growing tumor tissue.

## DISCUSSION

*N. langsdorffii* showed a free amino acid distribution which remained almost constant up to 150 r per day. When studying the gross morphology under irradiation conditions, this plant remained almost unaffected up to that dosage (4, 12). At 200 r per day there was a slight diminution in growth and a slight increase in axillary branch development. At 250, 325 and 400 r per day, however, the plant was reduced in height by one half or more, did not flower, and the leaves became increasingly linear. Thus, as the plant growth remained unaffected by radiation, free amino acid concentration remained constant, but just prior to morphological changes, increases in the acids occurred. After the onset of gross changes in the growth pattern, aspartic and glutamic acid decreased while glutamine and glycine continued to rise.

The hybrid showed changes in free amino acids

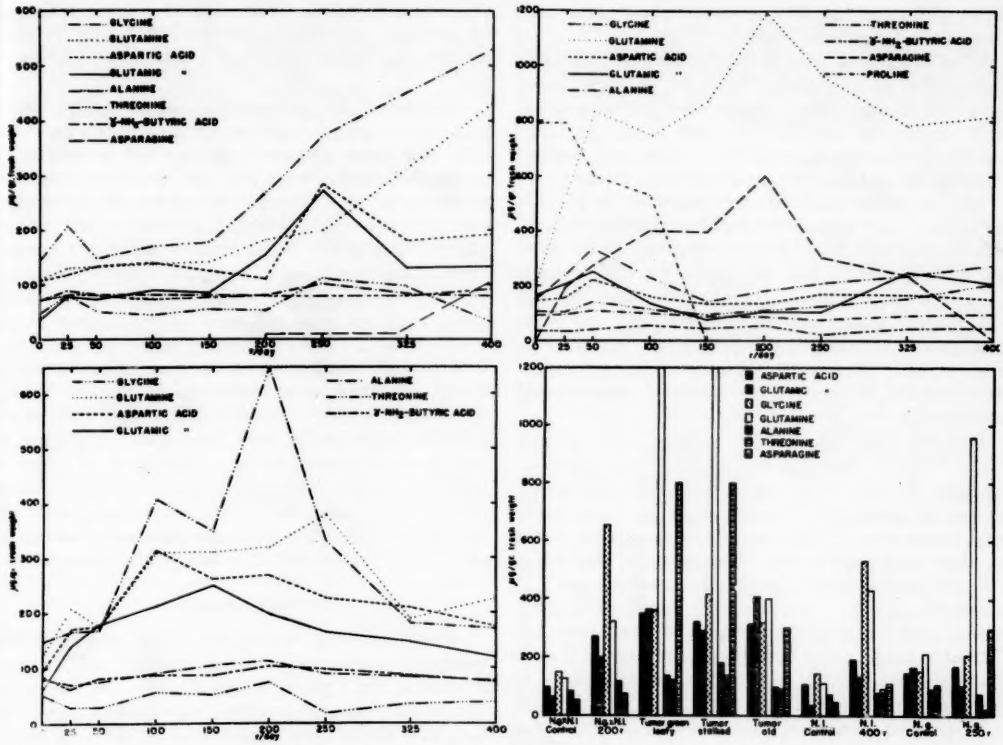


FIG. 1 (*upper left*). Concentration of free amino acids at 8 dosage levels and in control tissues, expressed as µg of the amino acid per gram fresh weight of the tissue, in the parent *N. langsdorffii*.

FIG. 2 (*lower left*). Concentration of free amino acids at 8 dosage levels and in control tissues, expressed as µg of the amino acid per gram fresh weight of the tissue, in the hybrid *N. glauca* × *N. langsdorffii*.

FIG. 3. (*upper right*). Concentration of free amino acids at 8 dosage levels and in control tissues, expressed as µg of the amino acid per gram fresh weight of the tissue, in the parent *N. glauca*.

FIG. 4 (*lower right*). Concentration of several amino acids in tissues of irradiated and non-irradiated tobacco plants, expressed as µg of the amino acid per gram fresh weight of the tissue. From left to right: *N. glauca* × *N. langsdorffii* control tissue, tissue high in amides—green leafy tumor, stalked tumor and old tumor; *N. landsdorffii*, control tissue and tissue high in amides; and *N. glauca*, control tissue and tissue high in amides.

much earlier than did the langsdorffii parent. When compared to control concentrations, significant increases in the free amino acids occurred at 100 r per day. At 250 r per day and above there was a drop in all acid concentrations. Morphologically, this plant began to show changes in growth pattern at dosages between 50 and 100 r per day, where slight diminution in size and axillary branching occurred. From 150 to 200 r per day the plants were much reduced in size, while at 250 r per day and above significant increases in tumor formation occurred in this hybrid. It appears, then, that with the onset of morphological changes in the plant, because of radiation, free amino acids began to accumulate in the leaves, particularly glutamine, glutamic acid, aspartic acid and glycine, and when tumor increase occurred, these free amino acids were substantially reduced in concentration in the leaves. If, however, the free amino acids of tumor tissue were also considered, then it was apparent that at the point where decreases in amino acids occurred in leaf tissue, the tumor tissue had high concentrations of these same acids.

The glauca parent showed an almost immediate increase in free amino acid content, except for proline. At 50 r per day most of the amino acids had risen to a peak above which they did not rise again, even at higher dosages. However, asparagine and glutamine continued to increase or remain high at 100, 150, 200, 250 and 325 r per day. Morphological studies have shown that this is the most sensitive of the three to radiation (3). Strap leaf formation, or leaf abnormalities, are evident at the same dosage levels at which free amino acid concentrations increase. It is also of interest to note that of the parents, it is the glauca under stress conditions which most closely approaches young tumor tissue free amino acid concentrations,

while the normal hybrid tissues are more nearly like those of the langsdorffii parent.

From the above data and previous morphological observations it is apparent that in all three plant groups the morphological changes which manifest themselves under this irradiation stress condition, have concomitantly, changes in the free amino acid content which closely parallel the gross structure changes.

An interesting similarity in response of the parents and hybrid is the glutamine/glutamic acid and asparagine/aspartic acid ratio change. Figues 1 and 3 show that when glutamine and asparagine increase, there is a corresponding decrease in glutamic and aspartic acid. Figure 2 does not show this response, but when tumor tissue is taken into consideration (fig 4) it is again evident that both glutamine and asparagine have increased far beyond the values of glutamic and aspartic acid.

Peterson and Naylor (9), working with tobacco plants, found that both frenching and pseudo-frenching (induced by maleic hydrazide treatment) resulted in significant increases in many of the amino acids found free in the tissues. It should be noted that here again one sees a ratio change. The glutamine/glutamic acid ratio in pseudo-frenching was 3 : 1 in controls and 9 : 1 in the maleic hydrazide treated. In frenching the same ratio was 0.8 : 1 in controls and 2 : 1 in frenched. The asparagine/aspartic acid ratio also increased—controls 3 : 1, maleic hydrazide 5 : 1; in frenched plants the controls were 2 : 1 and the diseased 4 : 1. In both parents, glauca and langsdorffii, where morphological effects are pronounced, the glutamine/glutamic acid ratios are 6 : 1 and 2 : 1 respectively while control tissues have 0.9 : 1 and 1 : 1. Asparagine/aspartic acid ratios at similar dosages are 5 : 1 and 0.5 : 1 while controls are both zero, since no asparagine was detected.

Steinberg (15) found that he could induce the frenching response in aseptically grown tobacco plants with the addition of certain amino acids, particularly isoleucine, to the nutrient culture medium. The imbalance in amino acids resulted in morphological changes similar to the frenching disease. He also found (16) that additions of bacterial diffusates would induce pseudo-frenching manifestations.

Numerous workers have shown that under a variety of stress conditions with various organisms, free amino acids have a tendency to accumulate. Thus, micronutrient deficiencies in tobacco (17), ultra-violet radiation of yeasts (14), potato tissue under aseptic conditions without coconut milk added (except for glycine, alanine and gamma-aminobutyric acid) and carrot tissue without coconut milk (18), and x-radiation treatment of yeasts (8) all resulted in the accumulation of free amino acids. It appears, therefore, that subjecting these organisms to several kinds of stress treatment (except nitrogen starvation, Steward and Pollard (18)) resulted in the accumulation of free amino acids. With mouse and rat tumor tissue, both solid and ascites, Kit and Awapara (6) indicated that these tumor tissues had measurably higher concentrations of free amino acids than did normal tissues. Christensen and Henderson (2) found that in mice tumors, free amino acids had a tendency to accumulate.

Because of the high concentrations of free amino acids in tumors produced on the hybrid it seems possible that these abnormal growths not only tend to accumulate amino acids but also that the acids thus accumulated may function in further tumor production. However, the problem remains — why do we have buildup of free amino acids under irradiation and other stress conditions? Are the responses similar? Do stresses represent an aging phenomenon? Usually aging in plants is accompanied by an increase in the amides glutamine and especially asparagine. Though both of these amino acids do increase in concentration during irradition, other amino acids at many dosage levels also increase. Additionally, tumor tissue shows high concentrations of both asparagine and glutamine, but tumor tissue which was known to be older in terms of length of time it had been growing showed decreases in both the amides. Further studies are necessary to elucidate both the mechanism and mode of action of radiation on free amino acid accumulation.

## Summary

Two Nicotiana species and their species hybrid were subjected to varying dosages of gamma-irradiation for a period of two months. By the end of this period leaf abnormalities were evident in all dosages with the *N. glauca* parent, above 50 r per day in the hybrid and above 150 r per day in the *N. langsdorffii* parent. In general where leaf abnormalities occurred there was a concomitant increase in the free amino acid content of these leaves. Similarities, both morphological and physiological, to other organisms under stress conditions are discussed.

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# CORRECTION TO THE PAPER ENTITLED "OSMOSIS AND DIFFUSION IN TISSUE: HALF-TIMES AND INTERNAL GRADIENTS"

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I am indebted to Dr. K. Glasziou for drawing my attention to a numerical error in this paper which originally appeared in Plant Physiol. 33: 275–278, 1958. The values of $\underline{a}$ given for the data of Buffel and of Ordin and Bonner should be 0.2 and 0.25, *not* 0.02 and 0.025. In consequence the calculated D-values should be $2.36 \times 10^{-5}$ and $2.26 \times 10^{-5}$ $cm^2 sec^{-1}$, respectively. The experimental D-values are thus about the same as that for self-diffusion in free water, and *not* about 1 % of this value, as stated in the paper. The other conclusions of the paper are not affected.

CHROMATOGRAPHIC TECHNIQUES: Clinical and Biochemical Applications—Ivor Smith, editor; Interscience Publishers, Inc., New York; and Interscience Publishers, Ltd., London. 309 pages. 1958. $6.75.

If it is admitted that discovery ranks high among the elements which contribute to scientific progress, then it follows that the experimental scientist should master the techniques which are conducive to discovery in his field of interest. The literature of the past 14 years offers ample evidence that—for physiologists and biochemists especially—paper chromatography serves not only as a powerful analytical tool but serves also as a potent aid to the discovery of new and unsuspected compounds in almost any biological material. Since awareness is a prerequisite to intelligent investigative exploitation, it is not unlikely that the serendipic role will outweigh the purely analytical in ultimate importance. It is therefore gratifying to find that Dr. Smith and his colleagues have made available a "how-to" book which is bound to have a profound beneficial effect upon both the quality and the *utility* of the chromatograms obtained by users of the technique—either prospective or accomplished—who will be guided by the suggestions in this excellent volume. This is a well-organized, readable book, obviously written by authors possessing a wealth of first-hand experience and not merely from a collection of references on filing cards.

Turning to the question of what the reader will find between the covers of this book, we note that 11 authors have contributed 17 chapters. All but two, Chapters 15 and 17, deal exclusively with paper chromatography, although the book's title might lead one to expect otherwise. In Chapter 15 (Steroids—R. W. H. Edwards) adsorption chromatography on alumina columns is also described, while in Chapter 17 (Model Experiments—I. Smith and J. B. Jepson) column chromatography is utilized for illustrative purposes.

Following a concise introductory chapter the editor divides the text into four main sections. Of these, the 1st, consisting of two chapters (Chapters 2 and 3, 53 pp.) undertakes a discussion of the chromatographic apparatus, the chromatographic routine, and of methods for desalting the sample when necessary. The 2nd section—comprising the bulk of the text (12 chapters, 212 pp.)—is devoted to the application of chromatographic techniques to specific classes of compounds. A basic strategy for the characterization of supervenient "spots" is outlined in the 3rd section (Chapter 16, 11 pp.). The final section (Chapter 17, 14 pp.) is essentially a laboratory manual containing instructions for a series of tested exercises designed to acquaint students with the basic principles of chromatography.

In the preceding paragraphs we have taken a broad look at the contents; let us now take a look at what the book is not from a similar vantage point. It is not—and was not intended to be—a comprehensive review of the literature. Consequently the bibliography is not extensive, but the authors, through a studied avoidance of bewilderment, will come closer to fulfilling their purpose. Plainly, they have practiced a discriminating restraint and have included only those methods, techniques and recipes which have been thoroughly tested at first-hand. The rare deviation from this principle is frankly acknowledged.

What special merit may each section have when viewed at closer focus? The general chapters of section one, dealing with the chromatographic system and its manipulation, can be recommended on the basis of thorough and systematic coverage of the steps and factors involved in the production of useful chromatograms. Although the directions are written for an apparatus of a particular design referred to as the "Universal Apparatus," the basic principles nevertheless can be applied to any system which may be available. The Universal Apparatus accommodates up to five 10-inch square sheets per run. Sheets of this size appear to be quite adequate for the examination of fluids and extracts obtained from animal sources. Proof of this can be seen in the photographs of many excellent chromatograms interspersed throughout the text. However in the reviewer's experience unfractionated plant extracts do not always behave as well on small sheets as they do on the standard $18 \times 22$ inch sheets. Why this is so is not entirely clear but the wide spread in the range of concentrations encountered in plant extracts and the complexity of the latter may be contributory factors. Space does not permit a more complete discussion of the phenomenon. The suitability of small sheets for a particular application can easily be determined by trial.

All of the location reagents described in the book have been formulated for application by dipping. A number of advantages over spray application are cited. Of these some are certainly valid but others seem to be mildly overdrawn. The reader has his choice. If he works with large sheets and has a well-designed air-driven sprayer at his disposal he will probably prefer spraying over dipping. In any event he will aver that the ideal method of application remains to be discovered. But the reader who may be looking for a wide variety of proven reagents will be gratified by what he finds in the various chapters. Not only have the authors assembled a powerful array of reagents of varying specificity but they have also worked out compatible sequential applications which permit much more information to be derived from each chromatogram.

In Chapter 3 Dr. Smith presents an illuminating discussion of desalting apparatus and techniques. Electrolytic desalting and its effect on the components of the sample receive extended treatment but not to the exclusion of other procedures. Also considered are electrodialysis, desalting by ion exchange, adsorption dialysis and solvent extraction. To effect deprotein-

ization of samples the author recommends and describes a simple, effective method for ultrafiltration. This chapter contributes importantly to the practical value of the book.

Each of the 12 chapters in section two describes chromatographic procedures for a related group of compounds. Almost all classes of popular interest to physiologists and biochemists are included; viz., amino acids, amines and related compounds; indoles; imidazoles; guanidines; purines, pyridines and their derivatives; sugars; keto acids; phenolic acids; acids of the citric acid cycle; barbiturates; cholesterol and its esters and, finally, steroids.

In the main these chapters follow a uniform pattern. Suitable solvents and location reagents, both general and specific, are described. Then, where applicable, special techniques, as for example, multiple dip sequences are presented. The final section of each chapter, devoted to clinical applications, will be of less interest to the plant physiologist or biochemist; but it will not get into his way should he choose to ignore this part. The tables of $R_f$ values, the maps and the photographs of actual chromatograms will undoubtedly prove to be of great value to anyone confronted with the problem of identifying unknown compounds. These chapters will certainly help the chromatographer to uncover new ones from plant extracts.

No book can be perfect and a reviewer could easily succumb to the temptation to expose all of the errors —if only to prove that he has perused it in its entirety. This book is no exception and errors do occur —most of them typographical and so minor. However, at least two deserve to be corrected in subsequent printings. In Table 4.3 the column under "Colour Reactions" contains the abbreviation, "I," obviously standing for isatin, but the symbol is not defined in the key on p. 63. On p. 56 the reader is promised that the question of desalting prior to isolation will be found on p. 274; this promise is not fulfilled.

For an overall estimate the reviewer can do no better than to quote the expert opinion of Dr. C. E. Dent who states in the Foreword ". . . this book will remain useful for a longer time than do most books written about rapidly evolving subjects, . . . ."—W. STEPKA, *Research Laboratory, The American Tobacco Company and Department of Physiology, Medical College of Virginia, Richmond, Virginia.*

THE PHYSIOLOGY OF FOREST TREES—Edited by Kenneth V. Thimann with the assistance of William B. Critchfield and Martin H. Zimmermann. The Ronald Press Company, New York. 678 pages. 1958. $12.00.

The present volume includes the papers and summarized discussion from the first International Symposium on Forest Tree Physiology which took place from April 8 to 12, 1957, at the Harvard Forest. The symposium, sponsored by the Cabot Foundation, attempted to organize the present knowledge on tree physiology and to indicate possible directions for further research. Thirty-three scientists participated, 21 of whom were from the United States, 5 from Canada, 4 from Great Britain, and one each from Norway, Germany, and New Zealand.

Thirty-five papers from 28 senior authors were contributed and have been included under 9 major subject headings. The 1st section, on water relations and sap movement, is the largest and contains 7 articles including a review on the rise of sap by Scholander. Scholander notes that many tree species in the vicinity of Oslo freeze during winter and as a consequence bubble seeding extends through the entire vascular system. Mechanisms for the repair of this seeding, other than by root pressure, are not known. A paper by Stone on dew absorption by conifer seedlings showed that with artificial dew, the needles remained alive from a resaturation of the needle tissue, while the roots were dying under conditions of soil drought. Other articles are on maple sap flow, rate and pattern of moisture movement in stems, tracheid structure, and nitrogenous compounds in tree xylem sap.

The 2nd section, on photosynthesis, includes a review article and a discussion by Huber of attempts which are being made to estimate photosynthesis under field conditions by measuring the $CO_2$ depletion at different atmospheric levels. Such techniques avoid the artificial climate in containers, although they are at a disadvantage in having to estimate the amount of $CO_2$ which is removed by turbulence to higher levels. A paper by Bormann demonstrated a change in photosynthetic efficiency from younger to older loblolly pine seedlings which gave an interesting correlation with natural stand conditions during each stage.

Part three, on general biochemistry, contains two articles on the distribution of some organic compounds among different taxa, a discussion of oleoresin exudation, and an article on some chemical processes in bark. The 4th section, on mineral nutrition, includes a discussion by Leyton of the possibilities and limitations in defining growth in relation to mineral ratios in conifer needles, and an article by Fraser on the use of tracers in determining the path and rate of isotope movement in tree stems.

Part five, on phloem transport, contains three articles including one on the use of aphids in sampling sieve tube sap, and a review by Zimmermann on translocation of organic substances in which a peak sucrose concentration is shown at progressively lower trunk levels during the night. The 6th section contains discussions on the relation of root growth to light, aeration, bud dormancy, and auxin secreted by mychorrhizae.

Parts 7 and 8 concern a miscellany of growth phenomena including the relation of growth to photoperiodism and thermoperiodism, and studies on cytoplasmic streaming, auxin activity, and tumors in white spruce. The work summarized by Kramer showed further evidence for a growth decrease in tree

seedlings under progressively higher night temperatures.

The last section, on reproduction, contains five articles, some morphologic in character, including an attempt by Stanley to determine the histochemical basis of strobili and vegetative bud differentiation in pine.

A valuable part of this volume is the discussion following each presented paper in which conclusions are often extended and clarified and research techniques carefully examined; some of the most noteworthy are those on suction pressure and on tree species which grow by sudden spurts (flushes). The excellence of these discussions prompts the suggestion that future Cabot symposia might include round tables with a subsequent published typescript of the proceedings.

The contributed papers demonstrate the extensive use which is being made at present of tree species in physiology and the ingenuity which is applied in this experimentation. Many of the studies were conducted in natural habitats or on material collected from natural habitats, and there is frequent reference to relationships with physical environmental factors. All contributions were from active research workers and much of the material had not been previously published or summarized. One criticism is that some articles are sketchy and resemble progress reports.

The printing is excellent and the text is well edited. This volume should be considered a necessary reference work for researchers in tree physiology, although it is in no sense a textbook, since much of the work presented is in the experimental stage or is highly specialized, and there are few summary or review articles.—J. R. Bray, *Dept, of Botany, University of Toronto, Toronto 5, Ontario.*

## Robert Bruce Withrow
### 1905–1958

Robert Bruce Withrow died on April 8, 1958 at the age of 53. With his passing this country lost one of its prominent photobiologists, and his collaborators a valued friend.

As botany undertakes the inquiry of molecular mechanisms, it requires a degree of precision that necessitates refinement of physical observation and measurement, and the introduction of physical analysis. It is in this region, defined loosely as biophysics, that the talents of Robert Withrow found particularly appropriate employ. For he appreciated deeply the operational techniques and limits of experimental physics, and was able to apply this knowledge to both fundamental and practical problems of plant behavior.

This appreciation and application is evident in the research papers of Withrow. Two works illustrate specifically Withrow's interest in bringing to Plant Physiology exactness and precision of expression. The first was "Radiant energy nomenclature," Plant Physiology 18: 476–487, 1943. This was revised and extended in his work "Generation, control, and measurement of visible and near-visible radiant energy," In: Radiation Biology, Vol. III. pp. 125–259. McGraw-Hill Co., 1956, which was prepared with his wife, Alice. It can be justifiably said that any investigation in photobiology—any experiment in which light is used quantitatively—should begin with a thorough study of this classic paper. Withrow's knowledge of radiation was recognized by the ASPP when the Society appointed him Chairman of the Committee on Physical Methods. He also served as the Society's secretary in 1945 and 1946 and vice-president in 1947.

Both in personal and professional life, altruism was a characteristic quality of Robert Withrow. If you asked him how he was, you would find yourself listening to the state of his wife's health, or about his daughter's recovery from an accident. He drove himself as does a child, and night would find him exhausted. Yet, in the press of work, he seemed never too busy to answer a question helpfully and charitably, or to aid in solving someone else's experimental problems. His technical proficiency and meticulous attention to detail were astonishing. He could tell you how to blacken brass screen uniformly, make fine platinum electrodes, or pour gealtin filters of a size or spectral characteristics that were not available commercially. He would extemporaneously design in detail an achromatic lens system or a capacitance micrometer—and the constructed apparatus differed but little from the original sketch. As a product of his association with the vacuum problems of the cyclotron at Purdue, where he taught physics during World War I, Withrow became acquainted with adhesives that would bind anything to anything. His practical and theoretical extrapolations of this knowledge were treasured by his associates.

Withrow obtained his undergraduate and master's degrees in electrical engineering in the University of Cincinnati in 1928. He continued work there as a research associate and became interested in the discrimination and measurement of light. The isolation of limited spectral regions was a topic of recurrent concern. His second publication, in 1930, was on this subject—"Cellophane and gelatine filters for the ultra-violet." This was extended in 1936 in "Dyed gelatine light filters," Trans. Illum. Eng. Soc. 31: 69–81, 1936 (with H. M. Benedict), and in 1953 in "Filters for the isolation of narrow regions in the visible and near-visible spectrum," Plant Physiology 28: 105–114, 1953 (with L. Price). The improvement and availability of interference filters fitted naturally with this interest. To this end, one of his most recent publications was on "An interference-filter monochromator system for the irradiation of biological material," Plant Physiology 32: 355–360, 1957.

The varied responses of plants to light fascinated Withrow. As a result, he moved to Purdue University in 1931 to become a research associate in Horti-

culture. In the next three years, several of his papers described the effect on plants of supplementing natural light with artificial illumination. Quite naturally this developed into an interest in photoperiodic responses. This interest was fostered by his marriage in 1931 to a plant physiologist, Alice Phillips, who shared fully in many of his research undertakings. One of his first papers on photoperiodism was "Photoperiodic responses of certain greenhouse annuals as influenced by intensity and wavelength of artificial light used to lengthen the daylight period," Plant Physiology 11: 225–249, 1936 (with H. M. Benedict). At this point, Withrow considered his background inadequate to pursue botanical research effectively. He spent the next two years working on his doctorate in plant physiology under Charles Shull at the University of Chicago. There he achieved the reputation of being an astute graduate student with unusual experimental ingenuity—an indefatigable worker whose final examination was outstandingly brilliant.

There followed a very active period of work by the Withrows, Robert and Alice, on the nature of the photoperiodic response. In particular, they delimited the effective spectral regions in "The effect of various wave bands of supplementary radiation on the photoperiodic responses of certain plants," Plant Physiology 15: 609–624, 1940, and examined the transport of the stimulus from leaves in "Translocation of the floral stimulus in Xanthium," Bot. Gaz. 104: 409–416, 1943.

In the evaluation of light responses, Withrow early realized the desirability of being able to grow thrifty plants without deficiency syndromes and with minimum variability. He turned his attention frequently to problems of nutriculture and published several papers on this subject. A synthesis of this research appeared in two manuals on "Nutriculture" (War Dept. Technical Manual, T.N. 20–500, and Purdue Univ. A.E.S., S.C. 328: 1–60). His knowledge of nutriculture was recognized by the U. S. Government, not only with respect to its military applications, but also in regard to the utilization of sub-marginal land for food production. To this end Withrow was sent on extra-territorial missions to Central and South America in 1945, and to the Near East and Europe in 1955 and in 1957.

On leave from Purdue University, Withrow spent 1947 and 1948 as a Senior Biophysicist at Argonne National Laboratory. He initiated a research group in plant radiobiology which focused principally on the movement of ions in tissues, and the influence of ionizing radiation on such movements. The Withrows then went to Washington, D. C. when Robert was appointed Chief of the Division of Radiation and Organisms of the Smithsonian Institution. The position was a perfect one to further his biophysical interests, and he soon was actively engaged in the extension of instrument design and the study of the relation of light to growth and form. How rapidly the work was progressing is shown by the contributions of Robert Withrow and his associates to the Annual Program of the Society at Stanford University in August of 1957. These were "Stimulation of protochlorophyll synthesis in dark-grown bean leaves by irradiation with low energy," "Kinetics of the far-red inactivation of photomorphogenesis in the bean hook," "Far-red radiant energy in modifying x-ray-induced chromatid aberrations in broad beans," and "The mode of action of light and temperature on the mechanisms of floral initiation." He had also been looking forward to undertaking another aspect of radiation research: the potentiation of x-ray effects in tumor therapy.

After the meeting at Sanford, Withrow served as chairman and guiding spirit of a symposium on "Photoperiodism and related phenomena in plants and animals," that was held at Gatlinburg, Tennessee, in October, 1957. Here, too, he was an active contributor and edited the papers that are to appear in a symposium volume. At this symposium the stress and excitement initiated a coronary attack. His recovery was apparently complete and he undertook again to lecture, inducing another and fatal recurrence.—Solon A. Gordon and Sterling B. Hendricks.

## Joseph Fisher Stanfield
### 1901–1958

The American Society of Plant Physiologists mourns the passing of Dr. Fisher Stanfield who died suddenly of a heart ailment on May 7, 1958. He was born at Lewisburg, Kentucky, June 4, 1901, the son of Charlie Grant and Mary Martha East Stanfield. He attended Western State College of Colorado where he obtained his A.B. degree in 1927. Dr. Stanfield subsequently attended the University of Colorado receiving the A.M. degree in 1930 after having taught biology for three years at the Littleton, Colorado, High School. He completed work for his Ph.D. degree in plant physiology at the State University of Iowa in 1937. On August 1, 1939 he married Miss Margaret Ellen Cox of Galesburg, Illinois.

Dr. Stanfield served as assistant professor of biology on the staff of Knox College, Galesburg, Illinois from 1931 to 1939. In 1939, he became a member of the Chicago Teachers College faculty where he served until 1943. In 1944 he became professor and head of botany at Miami University, Oxford, Ohio, where he remained until his death.

Dr. Stanfield had been a member of the American Society of Plant Physiologists for nearly 30 years. He became very active in its affairs in 1937 while in Chicago where he was associated with Professor Charles A. Shull in the editorial work of PLANT PHYSIOLOGY. Dr. Stanfield was appointed business manager of PLANT PHYSIOLOGY in 1941 and served as such until 1944. In 1944, Dr. Stanfield was appointed to the newly created position of Executive Secretary-Treasurer of the ASPP which he held until

his resignation in 1956. Dr. Stanfield did much to build up the financial resources and membership of the Society as well as the subscription list of the journal. He succeeded in expanding the society's membership and increasing the circulation of the journal in a remarkable way. Professor Shull writes, "Of the work that he did, I can speak only in the highest terms. He was always prompt and faithful. He took the hardest part of the work, and with skill and dispatch prepared about 10 of the more difficult papers for each number of the journal for approximately five years."

Professor Shull states that "In the work he had assumed, Dr. Stanfield had the faithful help of Mrs. Stanfield, who was enthusiastic about ASPP. No one could have given more unselfishly than they did to help ease the burdens that had become onerous indeed for me by that time. It is difficult for me to find words properly to express my gratitude for the assistance and for the gracious spirit in which the help was given. We were truly sorry when the cordial relationships we had established ended due to Dr. Stanfield's departure from Chicago."

The writer recalls Dr. Sanfield's personal satisfaction in reporting that the membership of the ASPP in 1956 had reached an all-time high and that the total assets of the organization exceeded any previous level even though expenses had been heavy due to greatly expanded activities of the society and journal. The gratification of Dr. and Mrs. Stanfield about ASPP affairs was an eloquent testimonial of their devotion to the society because both of them were ill at that time. It was largely because of this that Dr. Stanfield eventually felt constrained to relinquish his service as an officer of the American Society of Plant Physiologists in 1956. Mrs. Stanfield died soon afterwards. Her death was a tragic blow from which Dr. Stanfield never recovered. Professor Shull expresses the sentiments of all who knew them in saying, "They were lovely to know, and it was always a joy to work with them. It is a great sorrow that both of them have gone." Dr. Stanfield was a man of high ideals and courageous in his dedication to them. He possessed a fine capacity for sympathetic understanding of human nature in all walks of life. He was kindly and generous.

Professionally Dr. Stanfield was long interested in the physiology of reproduction in plants and particularly in the biochemical aspects of dioecism. His published reviews on sex in plants indicated a thorough knowledge of this field. He has published several important papers on sex in Lychnis and had research investigations in progress on this genus at the time of his death. He has also contributed several excellent papers on the physiological role of vitamins. Dr. Stanfield's abiding interest in teaching is evidenced by his many years of committee work and his publications on this subject. Dr. Stanfield was a member of numerous organizations having long been a Fellow of the AAAS and of the Ohio and Illinois Academies of Science. He was a member of and very active in the Botanical Society of America, American Association of Biology Teachers and the American Society for the Advancement of Education. He also held membership in the American Forestry Association, ASHS, ASPP, NEA as well as in Sigma Xi, Gamma Alpha, Phi Sigma.

Professor Stanfield's work for the society and its journal was begun in the early days of their existence. Due to his long tenure his work became fundamental and directional in character contributing greatly to the continuing success in service to science of the ASPP and PLANT PHYSIOLOGY. In profound gratitude the American Society of Plant Physiologists records this tribute to Dr. Stanfield.—W. F. LOEHWING.

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